



Aalborg Universitet

AALBORG UNIVERSITY
DENMARK

Peripheral and Central Effects of Morphine and the Impact of Methylnaltrexone in Human Experimental Pain Models

Brokjær, Anne

DOI (link to publication from Publisher):
[10.5278/vbn.phd.med.00020](https://doi.org/10.5278/vbn.phd.med.00020)

Publication date:
2015

Document Version
Publisher's PDF, also known as Version of record

[Link to publication from Aalborg University](#)

Citation for published version (APA):
Brokjær, A. (2015). *Peripheral and Central Effects of Morphine and the Impact of Methylnaltrexone in Human Experimental Pain Models*. Aalborg Universitetsforlag. Ph.d.-serien for Det Sundhedsvidenskabelige Fakultet, Aalborg Universitet <https://doi.org/10.5278/vbn.phd.med.00020>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal -

Take down policy

If you believe that this document breaches copyright please contact us at vbn@aub.aau.dk providing details, and we will remove access to the work immediately and investigate your claim.

PERIPHERAL AND CENTRAL EFFECTS OF MORPHINE AND THE IMPACT OF METHYLNALTREXONE IN HUMAN EXPERIMENTAL PAIN MODELS

**BY
ANNE BROKJÆR**

DISSERTATION SUBMITTED 2015



AALBORG UNIVERSITY
DENMARK

**Peripheral and Central Effects of Morphine
and the Impact of Methylnaltrexone
in Human Experimental Pain Models**

Ph.D. thesis by

Anne Brokjær

November 2014

Thesis submitted: November 7, 2014

PhD supervisor: Asbjørn Mohr Drewes

PhD committee: Pharm D, PhD, Associate Professor Parisa Gazerani (chairman)
Aalborg University

PhD, Associate Professor Anne-Marie Heegaard
University of Copenhagen

Professor, Dr.med. Frank Petzke
Universitätsklinikum Göttingen

PhD Series: Faculty of Medicine, Aalborg University

ISSN (online): 2246-1302
ISBN (online): 978-87-7112-276-3

Published by:
Aalborg University Press
Skjernvej 4A, 2nd floor
DK – 9220 Aalborg Ø
Phone: +45 99407140
aauf@forlag.aau.dk
forlag.aau.dk

© Copyright: Anne Brokjær

Printed in Denmark by Rosendahls, 2015

Acknowledgements	4
List of papers	6
Abbreviations	7
English summary	8
Danish summary	10
1 General introduction	12
1.1 Pain	12
1.1.2 Impact of pain	12
1.2 The pain system	13
1.2.1 The primary afferent	13
1.2.2 Sensory transmission	14
1.2.3 Innervation of the gastrointestinal tract	16
1.3 Pharmacological pain management	17
1.3.1 Opioids	17
1.3.2 Opioid antagonists	21
1.4 Experimental human pain models	22
1.4.1 Opioids in experimental pain models	22
1.4.2 Sex differences in pain perception	23
2 Objectives	24
2.1 Hypotheses and aims	24
2.1.1 Study I	24
2.1.2 Study IIa	25
2.1.3 Study IIb	25
3 Methods and key results	26
3.1 Study conduct	26
3.2 Study population	26
3.3 Study I	27
3.3.1 Methods	27
3.3.2 Key results	34
3.4 Study II	36
3.4.1 Methods	36
3.4.2 Key results	43
4 Discussion	45

4.1 Study I	45
4.2 Study IIa	46
4.3 Study IIb	48
4.4 Methodological considerations	48
4.4.1 Study population.....	48
4.4.2 Rectal drug administration.....	49
4.4.3 The experimental procedure	49
4.4.4 Study design	50
5 Conclusions	51
6 Future perspectives	52
7 Reference list	54

Acknowledgements

First and foremost I would like to express my sincere gratefulness to my principal supervisor Asbjørn Mohr Drewes and my daily supervisor Anne Estrup Olesen. I am indebted to Asbjørn Mohr Drewes for introducing me to research, for offering me a position as a Ph.D. student; and more importantly, for believing in me and for encouraging me to pursue an academic career. Although he frequently travelled the world as part of his professional duties, emails were always promptly answered and the same goes for review of my work. I highly appreciated this and the past three years has been a truly unique experience. My daily supervisor, Anne Estrup Olesen, possess' all the right qualifications for being the best supervisor one could wish for. This goes for both her professional- and indeed also her personal skills. I would like to thank her for her never ending encouragement, for looking after me and my interests, for guiding my in the right direction and for always having time to discuss little as well as big things.

I would like to thank my co-authors for their academic contributions, invaluable discussions and critical review of the papers: Albert Dahan, Carina Graversen, Mads Kreilgaard, Mikkel Gram, Lona L. Christrup and Ulrika SH Simonsson. A special thanks goes to, Mads Kreilgaard and Lona L. Christrup; Mads Kreilgaard for introducing me to population pharmacokinetics and pharmacology in general (and for his patience in doing so), for his invaluable contribution to paper I. He is further thanked for insightful discussions and supervision; for always having time to answer my numerous questions and for valuable feedback. Lona L. Christrup is thanked for sharing her knowledge, her insightful ideas to manuscripts and for productive discussions; and in addition, for her taking her time to provide valuable feedback on this thesis.

I would like to thank my dear colleagues at Mech-Sense, Department of Gastroenterology and Hepatology, Aalborg University Hospital for their assistance in different aspects of both the experimental studies and academia; and for providing an inspiring and caring working environment, both professionally and socially. You have all, with your specialized skills and personality, been invaluable to me. Particularly, I wish to thank research nurses Birgit Koch-Henriksen, Isabelle Myriam Larsen and Annie Baumwall for insightful assistance, enthusiasm and support in- and outside the laboratory, and Lecia Møller Nielsen for taking care of all aspects of the randomization procedure in study II. I also wish to thank Matias Nilsson who has been an immense help in providing me with beautiful graphical illustrations for my papers and this thesis. His artwork speaks for itself! I would also like to thank the staff at the Medical Library at Aalborg University Hospital for always being such a great help.

I wish to express my sincere thanks to the participants who bravely enrolled in the experimental studies – without their contributions this research project would not have been feasible.

The present Ph.D. project has only been possible through the financial support I have received from The Danish Council for Strategic Research, Det Obelske Familiefond, The Research Administration at Aalborg University Hospital (AFUA) and Heinrich Kopps Legat. I am extremely grateful for their contribution.

My family and friends (you know who you are) have showed me love and support; and have reminded me that life is more than achieving yet another goal in an academic career. I am lucky to have them in my life and I am them truly grateful. In particular, I would like to express my sincerest thanks and appreciation to my parents for their emotional support no matter what path I choose in life; this also goes for my brother and his family. In addition, I would like to express my genuine appreciation to my two nearest friends, Marianne and Annette, for knowing that I can always count on them. Their friendship has been and is invaluable to me. I also wish to thank Anton – for being a fantastic distraction, for always being happy and for his unconditional love. I regret that I have neglected my friends and family the past months but I know they understand my reasons for doing so.

Saving the most important for last, I would like to thank to my life partner, Kasper, for his unconditional love, patience, and continual support of my academic endeavours and for making me believe that everything will be okay. Aside from being my rock, he has also contributed academically to this thesis and my work in general. I am thankful for his genuine interest in my research, his academic insightfulness and for invaluable statistical discussions.

Anne Brokjær
November 2014

List of papers

The present thesis is based on two experimental studies performed in healthy male participants. Study I was carried out in the period of June 2012 to October 2012 and study II from September 2013 to April 2014, during my employment at Mech-Sense, Department of Gastroenterology & Hepatology, Aalborg University Hospital, Aalborg, Denmark. The experiments were conducted in the research laboratory at Department of Gastroenterology & Hepatology, Aalborg University Hospital. These two studies have given rise to three papers, which are listed below and the manuscripts are enclosed in the appendix. They are referred to as I, II and III in the text. Elements from study I, which has not been included in any of the papers, are also described in this thesis.

- I Brokjær A, Kreilgaard M, Olesen, AE, Simonsson USH, Christrup, LL, Dahan A & Drewes AM. Population Pharmacokinetics of Morphine and Morphine-6-Glucuronide following Rectal Administration - A Dose Escalation Study.
Submitted to European Journal of Pharmaceutical Sciences, August 2014
Invited for resubmission after minor revisions – resubmitted October 2014

- II Brokjær A, Olesen, AE, Christrup, LL, Dahan A & Drewes AM
The Effects of Morphine and Methylnaltrexone on Gastrointestinal Pain.
Submitted to Neurogastroenterology and Motility, October 2014

- III Brokjær A, Olesen, AE, Kreilgaard M, Graversen C, Gram M, Christrup, LL, Dahan A & Drewes AM. Objective Biomarkers of the Analgesic Response to Morphine in Experimental Pain Research
Will be submitted to Journal of Pharmacological and Toxicological Methods, November 2014

Abbreviations

cAMP	: Cyclic adenosine monophosphate
CNS	: Central nervous system
DRG	: Dorsal root ganglion
EEG	: Electroencephalography
ENS	: Enteric nervous system
GCP	: Good Clinical Practice
GI	: Gastrointestinal
ICH	: International Conference on Harmonisation
M6G	: Morphine-6-glucuronide
MNTX	: Methylnaltrexone
NS	: Nociceptive-specific
OIBD	: Opioid-induced bowel dysfunction
PAG	: Periaqueductal gray
PK	: Pharmacokinetics
RVM	: Rostral ventromedial medulla
UGT	: UDP-glucuronosyl-transferase
VAS	: Visual analogue scale
WDR	: Wide dynamic range

English summary

Evidence that opioids can produce analgesia outside the central nervous system has accumulated the past decades. However, in most human studies local administration of opioids in somatic tissue, especially the intra-articular injection of morphine, has been investigated. Thus, thorough information regarding local opioid analgesia on visceral peripheral opioid receptors in humans is lacking. Opioid antagonists are increasingly used to neutralize gastrointestinal side effects of opioids. However, antagonists may decrease the potential local analgesic effects of opioids exerted via peripheral opioid receptors in the gut. Thus, investigation of the effects of such antagonists is warranted. Altogether, this information may be clinically relevant, since pain originating from the gut is one of the most common reasons why patients are referred to gastrointestinal clinics. The overall objectives of this Ph.D. thesis were, by means of an experimental pain model, to assess the peripheral effects of morphine after local administration in the rectum and compare these peripherally mediated effects to the centrally mediated effects; and further to assess the peripheral effects of methylnaltrexone, a peripherally acting μ -antagonist (study II). Prior to this, a dose escalation study (study I) was performed to optimize the study design with regards to both dose and quantitative sensory tests, to assess the safety and tolerability and to attain more detailed information of the pharmacokinetic profile of morphine after rectal administration. The study population in both studies was healthy male participants.

Based on two different experimental pain studies, this Ph.D. thesis (1) assessed which of two quantitative sensory test that should be applied in study II; (2) assessed the safety and tolerability of three clinically relevant rectal doses of morphine; (3) evaluated an optimal morphine dose to use in study II; (4) described the population pharmacokinetics of rectally administered morphine; (5) investigated the peripheral effects of morphine before and after blocking of the peripheral μ -opioid receptors with the peripherally restricted μ -antagonist methylnaltrexone; (6) investigated the central analgesic effects of morphine; which included an assessment of the morphine effect on three objective measures (pupil diameter, prolactin secretion, and resting encephalography) and (7) compared the changes in these objective measures to subjective analgesia using a quantitative sensory test.

The findings from the dose escalation study showed, mechanical muscle stimulation was most sensitive to morphine analgesia compared with thermal skin stimulation; and that rectally administered liquid morphine, in the dosing range of 10-20 mg, was safe and well tolerated. A model was further developed to describe population pharmacokinetics of rectally administered morphine. The bioavailability was estimated to 24% which corresponds well to bioavailability after oral administered morphine. Based on the findings from this study, a morphine dose of 30 mg was considered to be optimal for study II.

No peripheral analgesic effect of morphine was found. On the other hand, methylnaltrexone may have exerted a local effect on rectal distensions and thereby appeared to improve analgesia. Methodological shortcomings in the experimental study design may have contributed to the lack of peripheral morphine

effects and thus, a peripheral effect of morphine on rectal pain during e.g., inflammation cannot be excluded. However, a 30 mg rectal morphine dose proved to be adequate to induce centrally mediated analgesia, which was manifested as an increase in tolerance to mechanical muscle pressure, a decrease in pupil diameter 30 minutes after dosing and an increase in prolactin concentration which followed 45 minutes after morphine administration. An effect was also seen on resting EEG, however it was considerable delayed compared to the onset of analgesia. Thus, only pupil diameter and prolactin concentration proved to be sensitive objective measures of central morphine analgesia. However, pupil diameter is the most feasible objective measure.

Danish summary

Gennem de seneste årtier har flere videnskabelige studier påvist, at opioider kan virke smertestillende udenfor centralnervesystemet. Humane studier har dog fortrinsvist undersøgt den lokale smertestillende effekt i somatiske væv. Viden om opioiders smertestillende effekt via opioid receptorer i tarmen er således mangelfuld. Opioid antagonist anvendes i stigende grad til at neutralisere gastrointestinale bivirkninger af opioider. Antagonister kan dog potentielt reducere en lokal smertestillende effekt af opioider via opioid receptorer i tarmen. En undersøgelse af effekterne af sådanne antagonist er således ønsket. Information om lokal smertestillende effekt kan være klinisk relevant, da smerter der stammer fra tarmen er en af de mest hyppige årsager til, at patienter henvises til gastroenterologiske afdelinger.

Det overordnede formål med dette Ph.D. projekt var, at undersøge den lokale smertestillende effekt af morfin efter administration i endetarmen samt at sammenligne disse lokale smertestillende effekter med smertestillende effekter medieret via centralnervesystemet. De lokale smertestillende effekter blev undersøgt før og efter blokering af opioid receptorer i tarmen. Til dette formål blev antagonist methylnaltrexon anvendt. For at undersøge de lokale smertestillende effekter af morfin, blev der udført et eksperimentelt smertestudie (studie II) der involverede anvendelse af forskellige kvantitative smertetest. Eksempelvis blev de lokale smertestillende effekter undersøgt ved hjælp af mekanisk smertestimulering af tarmen. Forud for dette studie, blev der udført et dosis eskaleringsstudie (studie I), der inkluderede to forskellige kvantitative smertetest, mekanisk muskel smerte og termisk hud smerte. Studie I blev udført for at optimere studiedesignet med hensyn til valg af både morfin dosis og smertetest samt for at vurdere sikkerheden i forbindelse med rektal administration af morfin. Et andet formål med dosis eskaleringsstudiet var, at opnå en mere detaljeret viden om morfins farmakokinetik efter rektal administration. Studie populationen var raske mandlige forsøgspersoner.

På baggrund af disse to eksperimentelle smerte studier, vurderede det nærværende Ph.d. projekt (1) hvilken af de to smertetest der skulle anvendes i studie II; (2) sikkerheden af tre klinisk relevante rektale morfin doser samt hvor godt forsøgspersonerne tolererede dem; (3) hvilken morfin dosis der skulle anvendes i studie II; (4) populations farmakokinetikken af rektalt administreret morfin; samt (5) undersøgte de lokale smertestillende effekter af morfin før og efter blokering af lokale opioid receptorer i tarmen med antagonist methylnaltrexon; (6) evaluerede morfins virkning i central nervesystemet; og herunder vurderede ændringerne i tre objektive mål for aktivering af centralnervesystemet (pupildiameter, prolactin sekretion og elektroencefalografi) og (7) sammenlignede ændringerne i disse objektive mål med den subjektive smertestillende effekt. Den subjektive smertestillende effekt blev undersøgt ved hjælp af en kvantitativ smertetest.

Resultaterne fra undersøgelserne viste, at morfin doser på 10-20 mg var sikre og veltolererede. Der blev udviklet en model til at beskrive populations farmakokinetikken efter rektal administration. Ud fra denne model blev biotilgængeligheden estimeret til 24 %, hvilket svarer til morfins biotilgængelighed efter oral

administration. Ud fra fundene i studie I blev det yderligere vurderet, at 1) mekanisk muskel smerte samt 2) morfin dosis på 30 mg skulle anvendes i studie II.

Der blev ikke fundet en lokal smertestillende effekt af morfin. Til gengæld kunne forsøgspersonerne tolerere mere smerte efter blokering af de lokale opioid receptorer. Methylnaltrexon syntes således at forbedre den smertestillende effekt. Metodologiske mangler i det eksperimentelle studie design kan have bidraget til den manglende lokale smertestillende effekt af morfin. En lokal smertestillende effekt kan derfor ikke udelukkes ud fra fundene i det nærværende studie.

En morfin dosis på 30 mg viste sig at være tilstrækkelig til at fremkalde centralt medieret smertestillende effekt. Dette manifesterede sig ved at forsøgspersonerne tolerede mere muskel smerte samtidig med at pupil diameteren blev mindre. For begge mål indtraf effekten 30 minutter efter dosering. Morfin inducerede også en stigning i prolactin koncentration 45 minutter efter dosering. Derfor blev der observeret en effekt på EEG, dog først 120 minutter efter dosering. Det var kun pupil diameter og prolactin koncentration der viste sig at være følsomme objektive mål for central morfin analgesi. Pupil diameter anbefales dog som objektive mål for aktivering i centralnervesystemet, når et simpelt objektive mål er ønsket.

1 General introduction

1.1 Pain

Pain is an essential function of the nervous system, which provides the body with a warning signal of potential or actual injury and is thus, an integral part of the defense mechanisms required for survival.

According to the taxonomy of International association for the study of pain (IASP), nociception is defined as: *“The neural process of encoding noxious stimuli”* whereas pain is defined as: *“An unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage”*(1). Pain is a subjective experience, it is always unpleasant, each individual learns the application of the word through own experiences and due to the unpleasantness of the pain, it also has an emotional aspect. Thus, it is a highly complex and multidimensional experience comprising three components: 1) The sensory-discriminative component, 2) The affective-motivational component and 3) The cognitive evaluative component (2). The sensory-discriminative component relates to the ability to locate pain, to identify the pain modality and to assess the intensity. The motivational-affective component on the other hand, considers the emotional aspect of the pain experience e.g. fear and anxiety. The cognitive evaluative component relates to the evaluation and interpretation of the pain experience. This is for instance influenced by anticipation and memory of the experience (2).

Pain is classified in nociceptive pain, neuropathic pain and pain due to sensitization, however only nociceptive pain is relevant in the context of this thesis. There are two distinct types of nociceptive pain; somatic and visceral. Somatic pain refers to pain that originates from skin, muscle or bone while visceral pain originates from internal organs (viscera). Visceral pain has several distinct characteristics that differentiate it from somatic pain. Somatic pain is well localized and well characterized by patients and in research in animal and humans. In contrast, visceral pain is deep, dull, diffuse and thus, poorly localized. In addition, visceral pain is accompanied by motor and autonomic reflexes e.g. nausea, vomiting and diarrhoea (3). Another particularity is that visceral pain can be referred to somatic structures remote from the stimulus (4). In contrast to somatic pain the mechanisms causing visceral pain are poorly understood. To optimize the treatment of visceral pain, it is essential to improve the present knowledge of the mechanisms causing pain and of the pharmacological mechanisms of action of analgesics.

1.1.2 Impact of pain

Considering the unpleasantness of the pain experience and the emotional aspect, it is not a surprise that chronic pain negatively affects many aspects of quality of life, daily activities, social and working lives (5,6) and patients suffering from chronic pain frequently display comorbid depression (7). According to a large survey (N=46394) performed in Europe and Israel, chronic pain of moderate to severe intensity affects 19%

of the adult population. Few patients suffering from chronic pain are managed by pain specialists and almost half do not receive adequate pain management (8).

Among the different types of pain, chronic pain originating from viscera is one of the most common causes of morbidity in the general population (4). Thus, chronic visceral pain represents an immense challenge for public health and constitutes a considerable socioeconomic burden as a result of medical expenses, hospitalization, and job absenteeism (5,6). The enticement for pain research is clear when considering the number of people suffering from chronic visceral pain.

1.2 The pain system

The somatosensory system is a complex sensory system, made up of a number of different receptors, including thermoreceptors, photoreceptors, mechanoreceptors and chemoreceptors. It can be divided into at least four distinct modalities: touch, proprioception, temperature and nociception. Each modality has its own receptors, fibers and pathways. Nociception begins in the periphery where sensory fibers detect mechanical, thermal or chemical changes above a certain threshold. Before pain can be perceived, the nociceptive signal goes through distinct processes referred to as transduction, transmission and modulation (9). Transduction is the process by which the stimuli (mechanical, thermal or chemical) is transduced into electrical energy and once the electrical energy reaches threshold value an action potential is induced. Transmission refers to transport of the signal from the periphery. The signal is passed along the axon of the neuron into the spinal cord and further to higher brain centres. Before reaching the cortical regions in the brain, modulation of the nociceptive signal takes place. Modulation will result in either an inhibition or a facilitation of the neuronal activity. Essentially, the nociceptive signal reaches the cortical regions where pain perception is generated (10-12).

1.2.1 The primary afferent

Primary afferent fibres are sensory neurons which respond to potentially harmful stimuli. Their cell bodies are located in the trigeminal (the face) and dorsal root ganglia (the body). Primary afferent fibres have a peripheral and central axonal branch; the peripheral axonal branch innervates their target organ, while the central axonal branch terminates in the dorsal horn of the spinal cord. The fibres terminate in so called “free nerve endings” where the noxious stimuli are transduced into electrical signals. The terminal end of the axonal branches is referred to as either the peripheral- or central terminals (11). There are three main types of sensory fibres in the periphery: A β -fibres, A δ -fibres and C-fibres. They have different properties (e.g. size, conduction velocity and presence/lack of myelin sheath) and can be characterized according to the type of stimuli they respond to e.g. chemical, thermal or pressure; or according to their tissue localization e.g. skin, muscle or viscera (10,12). These properties allow them to selectively respond and transmit sensory information of an intensity that is tissue threatening or damaging. The conduction velocity is directly

correlated to their diameter and degree of myelination (10-12). A β -fibres have large diameters and are highly myelinated. Thus, they conduct action potentials at high speed. Generally, these fibres have a low activation threshold and are responsible for the perception of non-noxious stimuli. Sensory fibres that respond to noxious stimuli (A δ - and C-fibres) are referred to as nociceptors. These fibres conduct relatively slowly, being only lightly myelinated or unmyelinated. Compared to A β -fibers, A δ -fibres have a smaller diameter, have higher thresholds for activation, are thinly myelinated and thus, are slower conducting. These fibres respond to chemical, thermal and mechanical stimuli (11). A δ -fibres are responsible for “first pain” which is rapid in onset, well localized and sharp or pricking character. The majority of C-fibres are polymodal and respond to thermal and mechanical stimuli while some C-fibres respond only to a specific type of stimuli. C-fibres have the highest thresholds for activation. Due to the light or non-myelination of the axon, these nociceptive fibres can only allow an action potential to travel at low speed, making them the slowest conducting nociceptors. As a result of their slow conduction velocity they are responsible for “second pain” which is characterized by a dull, diffuse and throbbing sensation (12). A group of unmyelinated nociceptors do not respond to stimuli unless they are activated by inflammation. They are usually referred to as “silent nociceptors” (11).

1.2.2 Sensory transmission

The recruited nociceptors (primary neurons) transmit the nociceptive signal from the periphery through the dorsal root ganglion to the dorsal horn of the spinal cord where the central terminal of the primary afferent fibres terminates. The dorsal horn is laminated in physiologically distinct layers (lamina) that extend from superficial to deep dorsal horn (11,12). Non-noxious A β -fibres primarily terminate in lamina III-V. C-fibres project superficially to lamina I-II while A δ -fibres project to lamina I and V (10,11) (Fig. 1). After reaching the designated lamina, the primary neurons project to secondary neurons and the first synaptic relay takes place (Fig. 2). In addition, the primary neurons make synaptic contact with inhibitory and excitatory interneurons in the dorsal horn. These interneurons are involved in the processing of the nociceptive signal before the secondary neuron project to higher brain centres. The secondary neurons can be divided into 1) nociceptive-specific (NS) neurons and 2) Wide-Dynamic-Range (WDR) neurons. NS neurons are primarily found in lamina I-II and respond to nociceptive stimulation mediated by A δ - and C-fibres or A δ -fibers alone. On the other hand, WDR neurons have the capacity to respond to both innocuous and noxious stimuli as they receive input from A β -, A δ - and C-fibres. Their response is graded according to stimulus intensity (9,12). Primary afferent neurons release a variety of neurotransmitters e.g. glutamate, substance P and calcitonin gene-related peptide from the central terminal. The released neurotransmitters mediate nociceptive signalling by activating receptors on secondary neurons in the dorsal horn (e.g. neurokinin, α -amoni-3-hydroxy-5-methyl-4-isoxazolepropionic acid and N-methyl-D-aspartat) (10). The received information is instantly projected by the secondary neurons to higher brain centres, primarily the thalamus.

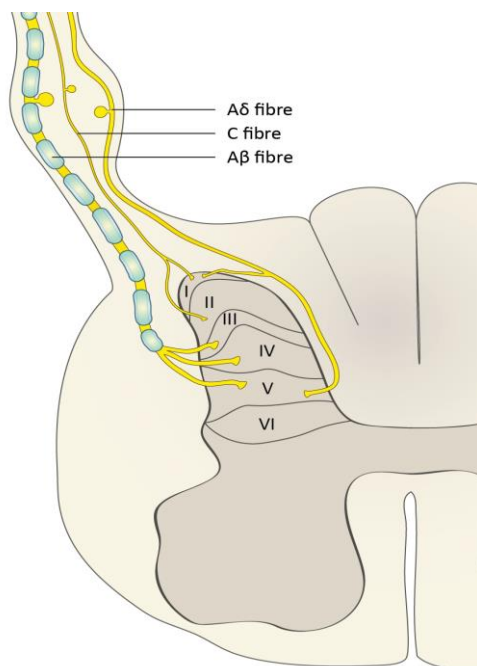


Fig. 1:

Recruited nociceptors transmit the nociceptive signal from the periphery through the dorsal root ganglion to the dorsal horn of the spinal cord. The dorsal horn is laminated in physiologically distinct layers (lamina) that extend from superficial to deep dorsal horn. Non-noxious A β -fibers principally terminate in lamina III-V. C-fibers project superficially to lamina I-II while A δ -fibers primarily project to lamina I and V.

For a large proportion of the afferents, a second synapse takes place in different nuclei of the thalamus. Secondary neurons may also make synapses with neurons in different nuclei of the brainstem – areas which are involved in endogenous mechanisms that inhibit or facilitate the nociceptive signal (descending modulation) (9). After these two synaptic relays, the tertiary neurons from the thalamus then project to the primary and the secondary somatosensory cortex, but also to limbic structures such as anterior cingulate cortex and amygdala (9,12). These cortical regions are collectively referred to as the “brain matrix” (13). Modulation of spinal nociception originates from various brain regions. The periaqueductal gray (PAG) and the rostral ventromedial medulla (RVM) are among the best described (14).

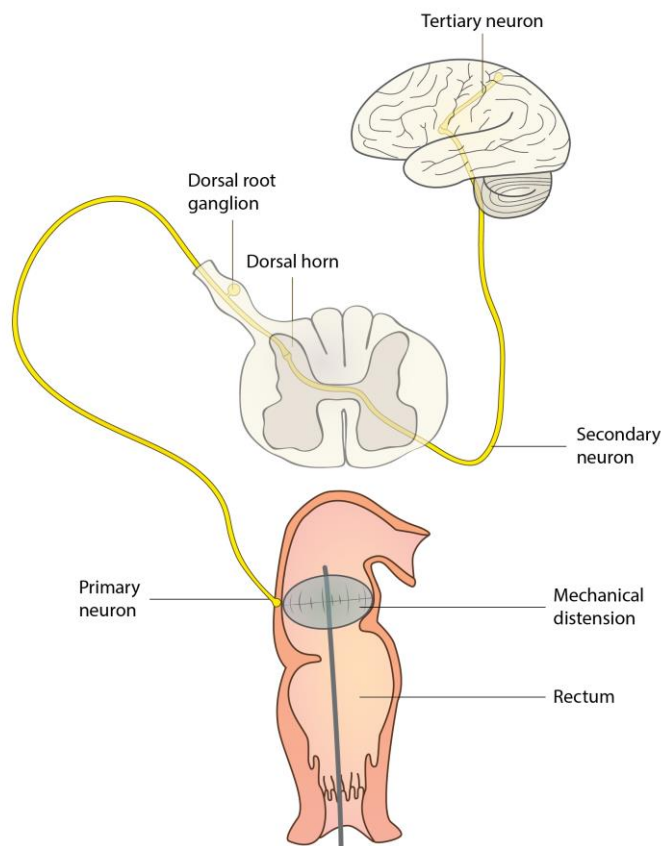


Fig. 2: Sensory transmission

Primary afferent nociceptors (primary neurons) in the periphery (e.g. visceral afferents) transmit the nociceptive signal from the periphery (e.g., pain following rectal distention) through the dorsal root ganglion to the dorsal horn of the spinal cord where the central terminal of the primary afferent fibers terminates. The primary neurons project to secondary neurons and the first synaptic relay takes place. The received information is instantly projected by the secondary neurons to higher brain centers. For a large proportion of the afferents, a second synapse takes place in different nuclei of the thalamus but also with different nuclei of the brainstem. Tertiary neurons from the thalamus then project to the primary and the secondary somatosensory cortex, but also to limbic structures

1.2.3 Innervation of the gastrointestinal tract

The gastrointestinal (GI) tract is innervated by sensory neurons in the central nervous system (CNS) (extrinsic afferents) as well as its own integrated network of sensory neurons (intrinsic primary afferent neurons), interneurons and motor neurons which form the enteric nervous system (ENS). The ENS is embedded in the gut wall – and extends from the esophagus to the anus. The ENS motor neurons can generally be divided into two groups: 1) muculomotor neurons that innervate the muscularis externa and muscularis mucosa; and 2) secretory neurons that innervate the intestinal secretory glands. Two major nervous plexuses; the myenteric and the submosal plexuses exist. The myenteric plexus is localized between the longitudinal and the circular muscle layers while the submucosal plexus, as indicated by the name, is located in the submucosa. These two plexuses are responsible of different functions; the myenteric plexus for controlling the motor activity within the gut while the submucosal plexus is responsible for secretory and absorptive activities (15,16).

1.3 Pharmacological pain management

Successful pain management depends on selecting the appropriate drug substance, the correct dose and administers it at the right time while carefully balancing analgesia against side effects. In an attempt to outline this, the “analgesic ladder” was introduced by the World Health Organization (WHO) (17). The analgesic ladder is a three step approach to the use of analgesics according to pain severity. The concept was originally intended for management of cancer pain; but has been extrapolated to non-malignant pain (9). The analgesic drugs should be introduced promptly when pain arise in increasing potency and titrated until the patient is free of pain. The approach is arranged in the following order: step 1) Non-opioid analgesics (aspirins or NSAIDs), then step 2) weak opioids (e.g., tramadol or codeine) and then if necessary step 3) strong opioids (e.g. morphine). Adjuvant drugs are recommended to calm fears and anxiety and to treat some pain disorders such as chronic pancreatitis and neuropathic pain. According to WHO, this drug strategy is inexpensive and 80-90% effective if the drugs are administered proactively every 3-6 hours as opposed to “on demand” administration. Non-opioid analgesics and adjuvant drugs are beyond the scope of the present thesis and thus, focus will on opioids, in particular morphine.

1.3.1 Opioids

The term “opiate” is used to describe naturally occurring alkaloids derived from the opium poppy plant. The term ”opioid” covers the description of all substances both endogenous and exogenous, which exert their actions on opioid receptors. Morphine is usually considered the “gold standard“ among exogeneous opioids. The opioid system consists of four opioid receptors (specific proteins): mu (μ), delta (δ), kappa (κ) and opioid-like receptor 1(18). Opioid-like receptor 1 displays 65 % sequence homology to the other receptors (19). All receptors are G-protein-coupled (G_i or G_o) and share the similar seven transmembrane helical structure with three intracellular and extracellular loops, an extracellular N-terminus and intracellular C-terminus. Opioid receptors are stimulated by endogenous opioids (i.e. endorphins, enkephalins and dynorphins) and can additionally be stimulated by clinically applied opioids (exogenous opioids). Most clinically relevant opioids exert their analgesic effect at the μ -receptor including the “gold standard” morphine. The majority of opioids are agonists, which interact with the opioid receptor and thereby produce an analgesic response. Pure agonists, such as morphine, are considered the most potent analgesics. Antagonists also bind to the opioid receptors, but exert no analgesic response and importantly, they can antagonize the effect of agonists.

1.3.1.1 Analgesic actions of opioids

Central opioid analgesia

All opioid receptor types are expressed on neurons in the dorsal horn of the spinal cord and it is a key area of opioid action (14). After opioid binding to receptors on presynaptic terminals of A δ - og C-fibers, a part of

the G-protein is released and it diffuses within the intracellular space to voltage gated ion-channels and to enzymes which can inhibit the voltage gated ion-channels. In brief, the comprehensive intracellular signalling involved in analgesia, essentially leads to an inhibition of calcium influx. This in turn leads to a decrease in the cyclic adenosine monophosphate. The overall effect is a reduced release of pain neurotransmitters such as glutamate, substance P and calcitonin gene-related peptide and thus, a decreased neuronal activity (Fig. 3). On postsynaptic terminals, the opioids inhibit potassium ion efflux which results in a decreased neuronal activity (18). Altogether, these processes ultimately lead to analgesia.

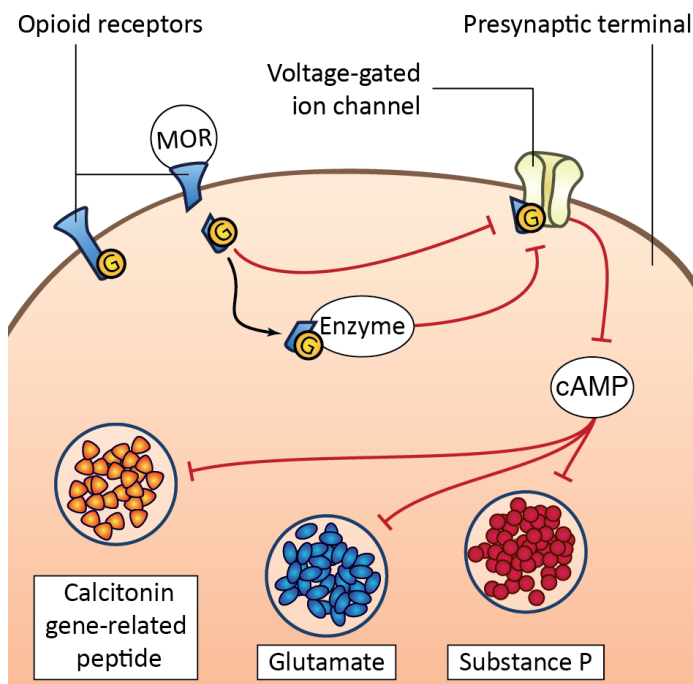


Fig. 3: Over simplified illustration of opioid mechanisms of action at the presynaptic terminal. After opioid binding to receptors on presynaptic terminals (e.g morphine (MOR)), a part of the G-protein is released. The G-protein diffuses to voltage gated ion-channels and to enzymes which can inhibit the voltage gated ion-channels. In brief, the comprehensive intracellular signalling involved in analgesia, essentially leads to an inhibition of voltage dependent calcium channels and thereby a decrease in calcium influx. This in turn leads to a decrease in the cyclic adenosine monophosphate (cAMP). Consequently, the overall effect is a reduced release of pain neurotransmitters such as glutamate, substance P and calcitonin gene-related peptide and thus, a decreased neuronal activity. Red lines indicate inhibition.

On the supraspinal level the most important and best studied sites of opioid action are the PAG and RVM although other supraspinal sites also support opioid analgesia (10,14). RVM sends projections to the dorsal horn along descending pathways. The μ -receptors are found in RVM where they play a principal role in opioid analgesia (14). The PAG sends projections to the RVM but does not project directly to the dorsal horn. Altogether, the projections terminate in the lamina relevant in the nociceptive processing. The net effect of these mechanisms is antinociception. The κ -receptors are distributed in the PAG although the primary opioid actions are produced by μ -receptors. Additionally, δ -receptors are found in the RVM, but their effects are not fully understood (18). The inhibition and the facilitation of the nociceptive transmission is the result of the activation on ON- and OFF cells in the RVM. When OFF-cells are activated it results in inhibition of pain while activation of ON-cells facilitates pain (14).

Peripheral opioid analgesia

For decades, opioids were thought to act only via interaction with supraspinal and spinal opioid receptors. However, this notion has been challenged and today it is well accepted that antinociception can not only be produced by opioid receptors located in the CNS, but also via opioid receptors on peripheral sensory neurons (20). The peripheral analgesic activity is exerted without activation of CNS opioid receptors and is thus devoid of centrally mediated side effects (see section 1.3.1.2). In addition it has been suggested that peripheral opioid analgesia is less sensitive to development of tolerance (21). It is well established that opioid receptors are synthesized in the DRG and are transported to central as well as peripheral nerve terminals (21). In humans, as in animals, opioid receptors are located on peripheral sensory neurons of small, medium and large size (22). Although opioids with a preference for μ -receptors are thought to be the most potent inducers of peripheral analgesia, δ - and κ -ligands are also believed to contribute (20). Evidence that peripheral antinociceptive effects may be mediated by peripheral opioid receptors has emerged in the past two decades. In humans, potent analgesic effects after local application have been reported after knee arthroscopy (23-26) and in patients undergoing dental surgery (27,28). However, the most extensively investigated local application is the intra-articular injection of morphine (reviewed in reference (29)). It has been demonstrated that peripheral analgesia is more pronounced in the presence of inflammation (30-34). An array of complex mechanisms has been suggested to contribute to the antinociceptive efficacy in inflamed tissue; although one major underlying mechanism is up-regulation of opioid receptors (20).

Peripheral opioid receptors (μ , δ and κ) have also been found in the gut, where they potentially exert a local effect on pain (35-37). Preclinical studies suggest that noxious visceral stimuli can be inhibited by κ -opioid agonists at the peripheral site (38-42). Supporting this; a novel peripheral restricted κ -agonist, ADL 01-0101 produced analgesia in chronic pancreatitis patients. The analgesic response was not associated with centrally mediated side effects, which support that the effects were peripherally mediated (43).

The discovery of opioid receptors in the periphery has stimulated research directed at developing peripherally active opioids without centrally mediated side effects. Hydrophilic compounds with restricted capability to penetrate the blood-brain-barrier have been common approaches. Examples of such compounds include the μ -agonist loperamide, which is originally intended for the treatment of diarrhoea, and asimadoline, a κ -agonist (20). The aforementioned peripheral restricted κ -agonist, ADL 01-0101, is an example of promising novel compounds (43). However, ADL 01-0101 has apparently not yet entered the market. Similarly, another novel peripheral restricted κ -agonist, CR665, had a selective effect on visceral pain, when compared with oxycodone in a multi-tissue experimental human pain model (44). However, centrally mediated side effects were reported, suggesting that CR665 is not fully peripherally restricted. The results from these studies support the hypothesis that opioid receptors are expressed in human visceral afferents and that analgesia can be produced in the periphery.

1.3.1.2 Opioid induced side effects

Opioid-induced analgesia can unfortunately be accompanied by an array of side effects. These side effects are related to the binding of receptors involved in other functions and are peripherally and/or centrally mediated (45). Common centrally mediated side effects include somnolence, sedation, mental clouding, mood effects (euphoria or dysphoria) and more rarely, respiratory depression. In addition, long-term use of opioid analgesics is associated with clinically relevant rates of abuse or addiction (46,47).

Binding of opioids to peripheral opioid receptors in the myenteric and submucosal plexuses in the gut results in increased tone, dysmotility, enhanced absorption of fluids and decreased fluid secretion. In the clinic, these effects are manifested as opioid-induced bowel dysfunction (OIBD), where symptoms such as reflux, abdominal distension and constipation are prevalent (15,16). OIBD rarely disappears during the course of treatment (48) and pharmacological interventions include laxatives (bulk agents, stool softener, stimulants, enemas) as first-line treatment. The next step in the treatment pathway is formulations containing opioid antagonists with effects restricted to the periphery (e.g. slow release naltrexone or methylnaltrexone) (16,49). Other common gastrointestinal side effects include nausea and vomiting (50) although, the majority of patients develop tolerance to the central mediated nausea and vomiting within the first week of treatment (48). Although opioids do provide effective analgesia for many, treatment outcomes are variable (51). For some patients, chronic treatment with opioids may be ineffective and the side effects may persist and even become intolerable. This hampers patient recovery and limits the clinical usefulness of the opioids (47,51).

1.3.1.3 Morphine

Morphine was the first natural opium alkaloid to be isolated from the opium poppy plant (*papaver somniferum*). Morphine is a μ -agonist and for decades morphine has been the most important analgesic drug to alleviate moderate to severe acute and chronic pain; and it remains the gold standard against which new (and existing) analgesic drugs are compared (52,53). Morphine can penetrate the blood-brain-barrier (a membrane that tightly segregates the brain from the blood) but due to its low lipid solubility, it passes slowly (53).

Administration

Morphine can be administered by different routes including oral, rectal, subcutaneous, intravenous, epidural and intrathecal routes. However, oral administration is considered mainstay for pain management due to its simplicity, convenience and economy (52). Oral administration is not viable in patients with conditions such as severe nausea, vomiting, dysphagia, gastrointestinal obstruction, intolerance to oral morphine or mental confusion and alternative routes of drug administration may be required (52,54). The rectal route offers an alternative to the oral route and is non-invasive, painless and cheap. The pharmacokinetics has been investigated after different rectal formulations (tablets, suppositories, solutions and hydrogel) (55-65).

Results from previous studies indicate that the level of analgesia achieved after rectal administration is comparable to that of oral morphine (56,61,64-66).

Metabolism, elimination and excretion

The major metabolic pathway is the formation of morphine-glucuronides and the liver is the principal site of biotransformation (52,53). The glucuronidation, which occurs rapidly after morphine enters the blood (18) stream, is mainly catalyzed by UDP-glucuronosyl-transferase (UGT) isoenzyme UGT2B7 (52). Morphine-3-glucuronide (approximately 50%) and morphine-6-glucuronide (approximately 10%) constitute the major metabolites (67). M3G is not considered to exert any analgesic actions; on the contrary M3G in high concentrations has been proposed to induce hyperalgesia (18,68). M6G has a strong affinity for the μ -receptor. In general, the literature supports that M6G mediates analgesic effects by activating μ -receptors (67). However, the contribution to analgesia after morphine administration varies across studies (69-71). M6G penetrates the blood-brain-barrier slower than does morphine. This is likely to be associated with the lower lipophilicity of M6G (67). The first-pass metabolism of morphine determines its systemic bioavailability. After oral administration, morphine is subject to an extensive first-pass metabolism and thus, resulting in a limited systemic bioavailability. The reported bioavailability after oral administration varies from 19% to 47% (53). The bioavailability after rectal administration has been reported to be 31-53 % depending on mode of administration (61,62,64). The elimination half-life of morphine is approximately 2 hours (52,53) and appears to be independent of administration route or formulation (53,72). Morphine and its metabolites are primarily eliminated via the kidney and excreted in urine, approximately 10% as unchanged morphine (52).

1.3.2 Opioid antagonists

Opioid antagonists act on opioid receptors and thereby antagonize the effect of agonists thus, ultimately preventing both the analgesia and side effects. Peripherally restricted μ -receptor antagonists, which only poorly penetrate the blood-brain-barrier (e.g. methylnaltrexone) or is fully first-pass metabolized in the liver (e.g. slow release naltrexone) have been marketed to prevent the gastrointestinal adverse effects (16,49). However, only methylnaltrexone is relevant in the context of this Ph.D. project. Methylnaltrexone is a peripherally restricted μ -opioid antagonist. It is a quaternary derivative of the antagonist naltrexone. Compared to naltrexone, methylnaltrexone has a greater polarity and lower lipid solubility as a result of adding a methyl group at the amine in its ring. Consequently, methylnaltrexone crosses the blood-brain-barrier poorly and therefore selectively blocks or reverses the undesired opioid induced peripherally mediated side effects in the gut without affecting centrally mediated analgesia (73,74). In the clinic, methylnaltrexone is used as a rescue medicine if standard laxative therapy has failed. It has been approved by European Medicines Agency (Europe) and Food and Drug Agency (USA) for treatment in adult patients

receiving palliative care (75). Methylnaltrexone is readily absorbed and C_{\max} is achieved approximately 0.5 hours after subcutaneous injection (76), although the clinical effect (defaecation) can be seen after 5-10 minutes (clinical observation). It has a plasma half life of approximately eight hours and is excreted via the kidneys and in the faeces. The most frequently observed adverse effects of methylnaltrexone are abdominal pain, nausea, flatulence and diarrhoea (74).

1.4 Experimental human pain models

As described in section 1.1, pain is a subjective experience and a highly individual perception. In patients suffering from chronic pain, the perception of pain is influenced by an array of factors including emotion, fear, anxiety, general malaise, cognitive responses and social consequences of the disease (2). Additionally, it has been demonstrated, that the pain experience does not correlate well with the severity of the pathological condition (77). Altogether, these individual psychological and physiological factors are a major shortcoming of clinical pain studies. Experimental pain models in healthy participants are advantageous as different pain modalities can be studied under standardized and reproducible laboratory conditions. This allow for a less confounded assessment (78). The main components of experimental pain models are 1) nociceptive stimulation and 2) the responses to these stimuli. In these models, the nature, intensity, frequency, location and duration of the stimulus can be controlled. The data obtained from experimental pain models are a psychophysical- and/or a biological response to the nociceptive stimulus (79). Different experimental stimulations have been established and the stimuli can be classified according to their physical properties into: electrical, thermal, mechanical, ischemic and chemical pain stimuli and stimulation can be performed in various tissues including muscles, bones, skin and viscera (reviewed in reference (80)). Stimulation modalities relevant for the present Ph.D. project are described in section 3.

The evoked pain sensation can be quantitatively assessed with subjective methods such as visual analogue scale (VAS), numerical rating scales or pain thresholds and qualitatively, e.g. by applying the McGill Pain Questionnaire (80,81). The stimulus-response relationship can hereby be investigated. The VAS is generally used for the sensory dimension of the pain experience. To assess the pain pathways or to elucidate underlying pain mechanisms, the subjective methods can be combined with objective methods such as the nociceptive withdrawal reflex, resting electroencephalography (EEG), cerebral evoked potentials and imaging techniques (e.g. fMRI and PET/SPECT) (80).

1.4.1 Opioids in experimental pain models

The induced pain stimulus can be modulated by administering an analgesic drug and assessment of the analgesic effect can subsequently be performed. To evaluate the analgesic effect in experimental pain studies, the following factors are to be considered when designing the trial: 1) The model, 2) the dose and 3) the dosing regimen and timing. Firstly, the model needs to activate the mechanisms and pain pathways

relevant for the given analgesic drug, and secondly, a drug dose that balances the analgesic effect and side effects is essential. Thirdly, it is relevant to consider the dosing regimen i.e. whether single or multiple doses are preferred (80,81). In addition, relevant time points for testing should be selected on the basis of the pharmacokinetic (PK) profile of the analgesic (80). If an opioid is the analgesic in question, as in study I and II, it is essential to bear in mind that they must cross the blood-brain-barrier and enter the CNS to exert their (primary) analgesic effect resulting in a lag-time to the onset of analgesia (81). Importantly, it is preferable to use relatively high doses when analgesic effects are investigated (80).

1.4.2 Sex differences in pain perception

The literature points towards differences in pain perception among men and women. On average, women tend to report more intense pain, longer pain duration and more frequent pain (82-84). Sex differences in perception of pain have been demonstrated in clinical and as well as in experimental settings (85,86). Possible explanatory factors for these differences may include biological, psychological, and cultural differences, divergent social role expectations and an individual's past history (87). Biological sex differences, such as hormone variability, may provide a partial explanation to the observed sex differences in the response to experimental pain (88). Thus, the effect of the menstrual cycle on pain perception cannot be ignored. In experimental pain studies evoking somatic as well as visceral pain, females should therefore be examined in the same part of the menstrual cycle. This can be logistically challenging and further prolong the experimental study. Consequently, male participants are generally preferred over female participants. This was the case in the present Ph.D. project.

2 Objectives

Evidence that opioids can exert analgesia outside the CNS has accumulated the past decades. However, in most of the human studies, local administration of opioids in somatic tissue has been investigated. This is particularly true for intra-articular injection of morphine. Thus, in-depth information regarding local opioid analgesia on visceral peripheral opioid receptors in humans is lacking. Opioid antagonists are increasingly used to neutralize gastrointestinal side effects of opioids. However, antagonists may decrease the potential local analgesic effects of opioids exerted via peripheral opioid receptors in the gut. Thus, investigation of the effects of antagonists is warranted. Altogether, this information may be clinically relevant, since pain originating from the gut is one of the most common reasons why patients are referred to gastrointestinal clinics. Thus, the overall objectives of this Ph.D. thesis were to assess the peripheral effects of morphine after local administration in the rectum and compare these peripherally mediated effects to the centrally mediated effects, and further to assess the effects of the peripherally acting μ -antagonist MNTX after subcutaneous administration. To optimize the study design (study II) and to attain more detailed information of the PK profile of morphine and M6G after rectal (liquid) administration, a dose escalation study (study I) was performed.

2.1 Hypotheses and aims

2.1.1 Study I

It was hypothesized 1) that the dose escalation study with three clinically relevant doses that included two experimental pain stimuli would assess which of two quantitative sensory test, that would be most sensitive to morphine analgesia and therefore qualify to be used in study II; 2) that the three chosen doses of 10, 15 and 20 mg morphine would prove to be well tolerated and safe; 3) that an optimal morphine dose for administration in study II could be selected on the basis of the results of study I, and finally that 4) population PK modelling would provide a more detailed understanding of the pharmacokinetic profile of morphine and M6G after rectal administration of morphine.

The aims were therefore:

- 1) To evaluate which of two quantitative sensory tests that should be applied in study II
- 2) To assess the tolerability and safety of three clinically relevant doses of rectally applied morphine
- 3) To evaluate an optimal morphine dose for study II
- 4) To develop a population PK model of liquid rectal morphine and M6G

2.1.2 Study IIa

It was hypothesized that an experimental model including both visceral and somatic stimulations would 1) enable differentiation between local visceral and central somatic effects after rectal administration of morphine and 2) demonstrate whether MNTX interacts with the local visceral effects.

Accordingly the aims were:

- 1) To investigate the peripheral effects of morphine before and after blocking of the peripheral μ -opioid receptors with MNTX
- 2) To investigate the central effects of morphine

2.1.3 Study IIb

It was hypothesized that pupil diameter, prolactin concentrations and resting EEG would yield different sensitivity to morphine.

The aims were therefore:

- 1) To assess the effect of morphine administration on pupil diameter, prolactin secretion, and resting EEG
- 2) Compare the changes in these objective measures to subjective analgesia using mechanically evoked muscle pain.

3 Methods and key results

Section 3.1 and 3.2 gives a general introduction to the study conduct and the study population of both study I and II. This is followed by methods (section 3.3.1) and key results (section 3.3.2) for study I which is followed by methods (section 3.4.1) and key results (section 3.4.2) for study II. In the method sections, background information and rationale related to the different methods are described.

3.1 Study conduct

The present thesis is based on data from two experimental pain studies, referred to as study I and II. The studies were conducted in the research laboratory of Mech-Sense in Department of Gastroenterology & Hepatology, Aalborg University Hospital. Participants were informed in writing and then verbally before deciding to participate. After enrolment, at a separate screening session, the participants were instructed in and accustomed to the laboratory setting and the comprehensive pain testing procedures including the use of pain rating scales. These instructions were reinforced before each experimental test throughout the experiment using specific phrases to maximize reproducibility. To minimize bias, the experimental procedures were performed by the same two investigators. The experiments were conducted under controlled and quiet laboratory conditions devoid of interruptions and other disturbing factors.

Both studies were included in the same protocol. The protocol and the informed consent form were approved by The North Denmark Region Committee on Health Research Ethics, Denmark (N-20110077), The Danish Health and Medicines Authority (EudraCT identifier: 201100516920). It was further registered in clinicaltrialsregister.eu. The study was conducted in accordance with the Declaration of Helsinki and International Conference on Harmonisation (ICH) Good Clinical Practice (GCP) guidelines. GCP guidelines are the ethical and scientific standards developed to guide researchers in conducting biomedical research. GCP is applied to all studies involving humans, regardless of the risk. These standards are intended to ensure that the rights and the welfare of the participant are protected and further; that the research study is scientifically sound (89). The study was monitored by the GCP unit at Aarhus and Aalborg University Hospitals.

3.2 Study population

Study I and II were both performed in healthy male participants of Northern European descent aged 18 to 65 years (n=10 and n=15, respectively). Their health was verified by a thorough medical history and physical examination (including measurement of blood pressure and oxygen saturation). The examination was performed by a medical doctor. To be eligible for enrolment, it was further essential that the investigator was convinced that the participant: 1) Fully understood the contents of the study; 2) Was willing and able to comply with instructions; 3) Had the possibility to attend all the different experiments; and 4) Intended to

complete the study. Exclusion criteria were: 1) Allergy to study medication; 2) Participation in other medical studies fourteen days prior to the screening session; 3) Present and/or previous addictive behaviour observed in the participant or first degree relatives. Addictive behaviour is defined as abuse of alcohol, marijuana, opioids or other narcotics; 4) Present or previous chronic and/or pain related diseases; 5) Previous and/or present psychiatric diseases; 6) An expected need of medical treatment, surgery or hospitalization within the timeframe of the study; 7) Use of opioid analgesics and 8) Use of any type of analgesics 24 hours prior to a study visits.

3.3 Study I

3.3.1 Methods

Study I was an open label, cross-over study with escalating doses of morphine hydrochloride. The study had four separate treatment arms, which were separated by a minimum of one week washout period. At the first study day, 2 mg morphine hydrochloride was administered intravenously. Intravenous administration of morphine allows for determination of the proportion of the rectal dose that is absorbed and thus, for calculation of the absolute bioavailability. If rectal morphine administration is warranted in the clinic, it is generally formulated as suppositories. However, the rate of dissolution or melting of the suppository, and hence the rate of absorption is highly variable between individuals. Consequently, a liquid formulation, where morphine is readily available for absorption, was applied in the present study. For safety reasons a 10 mg dose was chosen as a start dose since the few published studies of liquid rectal administration (in adults) have used similar doses (59-61,90) (see Table 1, page 53). Study visits, dose escalations and mode of administration are summarized in Table 2. As a safety precaution the dose escalation was terminated if unacceptable adverse effects occurred in at least two participants. In this case, the dose would be adjusted to a lower dose; for example, if unacceptable adverse events were induced after administration of 20 mg rectal liquid morphine, a 15 mg morphine dose was thus to be used in study II. Unacceptable adverse effects were defined as unacceptable nausea, vomiting, dizziness, respiration depression or sedation. This assessment was conducted by a medical doctor.

Table 2: Overview of study visits, mode of administration and doses.

Study visit	Mode of administration	Drug	Dose
1	Intravenous	Morphine Hydrochloride	2 mg
2	Rectal	Morphine Hydrochloride	10 mg
3	Rectal	Morphine Hydrochloride	15 mg
4	Rectal	Morphine Hydrochloride	20 mg

3.3.1.1 Rectal morphine administration – a custom-designed approach

The principal mechanism of drug absorption from the rectum is passive diffusion. Drug will be absorbed in the submucosal venous plexus, which is drained by the superior, the middle and the inferior rectal veins (91). In the upper rectum, the superior rectal vein drains via the portal system and morphine will undergo first-pass metabolism before entering the systemic circulation. The lower part of the rectum is drained by the middle and inferior veins, which drains into the systemic system via the internal iliac vein. However, as extensive anastomosis exists between these two systems, only a partial avoidance of hepatic first-pass metabolism can be achieved (92,93). Altogether these mechanisms were considered, when designing study I and II. In addition, to avoid adsorption of morphine to faeces, the participants had their bowels cleansed before drug administration. Drug administration was performed in the upper rectum, 20 cm from the anal verge. This site was preferable to the lower rectum, as morphine would go via the portal system before entering the systemic circulation and thereby, a wider “peripheral time frame” could be attained. The peripheral timeframe was defined as the timeframe from drug administration until the drug exerted a measurable effect mediated via the CNS. For drug administration, a custom-made rectal probe was developed for study I. The probe had a length of 25 cm and four lumens for medicine administration. In addition, one lumen contained a guide wire to stabilize the probe. The corresponding medicine outlets were positioned one cm below the tip of the probe. The probe enabled medication to be sprayed evenly onto the rectosigmoid wall.

3.3.1.2 Population pharmacokinetic modelling

Pharmacokinetics (PK) is the quantitative study of what the body does to a drug substance and describes the absorption, distribution, metabolism and elimination (rate processes) (94). In brief, a PK model describes the mathematical relationship between the administered dose and the observed drug concentration-time profiles in an individual. The concentration should ideally be measured at the effect site (biophase), where the interaction with the receptor system occurs. However, in general this is not possible when dealing with drugs which exert their effects within the CNS. Most often the concentration is measured in more easily accessible body fluids such as blood as the case in the present study (95). The structure of a PK model can be illustrated by a diagram (boxes interconnected with arrows, see Fig. 6), which describes the rate of drug transfer between various compartments. Mathematically, the model can be described in terms of compartment volumes, clearances, rate constants etc. (94,95).

Analysis of PK data using a modelling approach is typically done by either an individual two-stage approach or a population approach, to describe typical parameters for the population and the variability. The population approach (also called non-linear mixed effect) has the strength of using all data simultaneously and thereby informing the model with each of the individual profiles to compute the typical population profile in addition to describing the variability in the data. Non-linear *mixed effect* modelling encompasses

fixed and random effects (95). Fixed effects are the typical parameters for the population (also called the structural model). Random effects describe the variability from the population mean, in terms of inter-individual and inter-occasion variability in addition to remaining residual error in the model. Description of the population variability is important to identify factors (e.g. demographics or genetics) that may help understand why some patient subgroups differ in their clinical response from the ‘typical’ population. These factors are termed covariates in a population model. To attain a sound description of the PK, it is essential to draw adequate blood samples during the time course (94). Each participant had one pre-dose and nine post-dose blood samples drawn. The sampling times were chosen on the basis of the PK of a previous study of rectal liquid morphine administration (61). To obtain detailed temporal PK information a sampling matrix design was applied. According to the sampling matrix design, participants were allocated to individual blood sampling times. This resulted in the following post dose sampling intervals: 1-5 minutes, 6-10 minutes, 11-15 minutes, 16-20 minutes, 22-30 minutes, 36-60 minutes, 66-90 minutes, 96-120 minutes and finally 130-180 minutes (Table 3).

Table 3: Overview of the applied sampling matrix. Each individual was identified by a unique identification number (ID) (001-010). Identification numbers are highlighted in black. The allocated individual sampling times (time 0-9) are given in minutes in the columns below the identification numbers.

SAMPLING MATRIX DESIGN										
ID	001	002	003	004	005	006	007	008	009	010
Time 0	0	0	0	0	0	0	0	0	0	0
Time 1	1	2	3	4	5	1	2	3	4	5
Time 2	6	7	8	9	10	6	7	8	9	10
Time 3	11	12	13	14	15	11	12	13	14	15
Time 4	16	17	18	19	20	16	17	18	19	20
Time 5	22	24	26	28	30	22	24	26	28	30
Time 6	36	42	48	54	60	36	42	48	54	60
Time 7	66	72	78	84	90	66	72	78	84	90
Time 8	96	102	108	114	120	96	102	108	114	120
Time 9	130	140	150	160	180	130	140	150	160	180

Each participant had one blood sample drawn within each of the intervals. The time differentiation scheme allowed for additional points on the PK curve than if each participant had been allocated to the identical sampling times. NONMEM 7.2 (ICON Development Solutions, Hanover, Maryland, USA) were used for the population PK modelling. All intravenous and rectal serum concentration-time profiles of morphine and M6G were modelled simultaneously. For detailed information regarding the bioanalytical analysis and the population-PK analysis, the reader is referred to (I).

3.3.1.3 Subjective pain assessment

Psychophysical methods can be applied to quantitatively assess pain perception in participants. They are based on the subjective pain experience and are measured by means of either standardized scales or as pain thresholds. A combined electronic visual analogue scale (VAS) for measurement of non-painful (0 to 5) and painful (5 to 10) sensations was used to assess the pain perception (Table 4). The VAS was combined with anchor words to describe the sensations: 0 = no perception, 1 = vague perception of mild sensation, 2 = definite perception of mild sensation, 3 = vague perception of moderate sensation, 4 = definite perception of moderate sensation, 5 = the pain threshold, 6 = mild pain, 7 = moderate pain, 8 = pain of medium intensity, 9 = intense pain, and 10 = unbearable pain. The VAS has proven to be robust and valid in assessment of experimental somatic (96) and visceral pain (97). Moderate pain (VAS=7) was chosen as the stimulation endpoint for the thermal skin stimulation and the mechanical muscle stimulation.

Table 4: The combined VAS with anchor words

Combined VAS with anchor words	
0	No perception
1	Vague perception of mild sensation
2	Definite perception of mild sensation
3	Vague perception of moderate sensation
4	Definite perception of moderate sensation
5	The pain threshold
6	Mild pain
7	Moderate pain
8	Pain of medium intensity
9	Intense pain
10	Unbearable pain

3.3.1.4 Pain stimulation

The analgesic effect of morphine on two quantitative sensory testing measures, mechanical muscle stimulation and thermal skin stimulation was assessed in the present study. The purpose of this assessment was to evaluate which was the most sensitive to morphine analgesia and thus, which to be applied in study II. For both methods, stimulations were performed at baseline and eight times after dosing according to a matrix similar to the blood sampling matrix: This resulted in the following eight post dose pain stimulation intervals: 2-6 minutes, 8-12 minutes, 14-18 minutes, 21-25 minutes, 30-54 minutes, 60-84 minutes, 90-114 minutes and 130-180 minutes. Stimulations were performed in the indicated order: 1) mechanical muscle stimulation and 2) thermal skin stimulation.

Mechanical muscle stimulation

Pain from muscles is clinically manifested as a cramp-like, aching and diffuse sensation. It may also be associated with referred to other deep somatic tissue. A δ - and C-fibers are primarily located in wall of arterioles of the muscle belly (98). Experimental muscle pain models can be divided into exogenous or endogenous models. In endogenous models the pain is induced by natural stimuli e.g. by exercise induced pain or ischemic pain whereas external pain stimuli (e.g. mechanical, electrical or chemical induced pain) is used in exogenous models (99,100). An exogenous model by means of the pneumatic tourniquet cuff (cuff) (101,102) was applied (Fig. 4). The cuff enabled mechanical stimulation of gastrocnemius muscle of the right leg with pressure being uniformly delivered to deep as well as superficial tissues. This stimulation method has successfully been employed in pharmacological testing (103,104) and has proven to be both reliable and sensitive (101).

Thermal skin stimulation

In most studies of analgesic actions, experimental models in skin have been used, which is likely due to the easy access (80). The skin is innervated by cutaneous nerve fibres sensitive to heat, cold, pressure, irritation, itch and pain. Cutaneous nociceptors are found in the dermis or epidermis and include A δ - and C-fibers, although unmyelinated fibers accounts for approximately 90% of all cutaneous nerve fibers (105).

Thermal skin stimulation is one method and it can be performed by cold and heat pain. Heat pain was used in the present study and was performed by means of a heating thermode (TSA II, MEDOC, Ramat Yishai, Israel) sized 25x50 mm (Fig. 4). It was applied 10 cm from the elbow joint on the volar surface of the left forearm. Thermal stimulation can be achieved with a rapid or a slow rate. Heating with a slow rate of (1° C /sec) was used in the present study. The temperature increased from 32° C to 52° C. A temperature of 52° C was chosen as the stimulation endpoint due to safety reasons. The slow rate was used as it predominantly activates C-fibers (14) and consequently, is suitable for detection of opioid effects as it is the traditional opinion that opioid mainly attenuate C-fibre mediated pain (80).

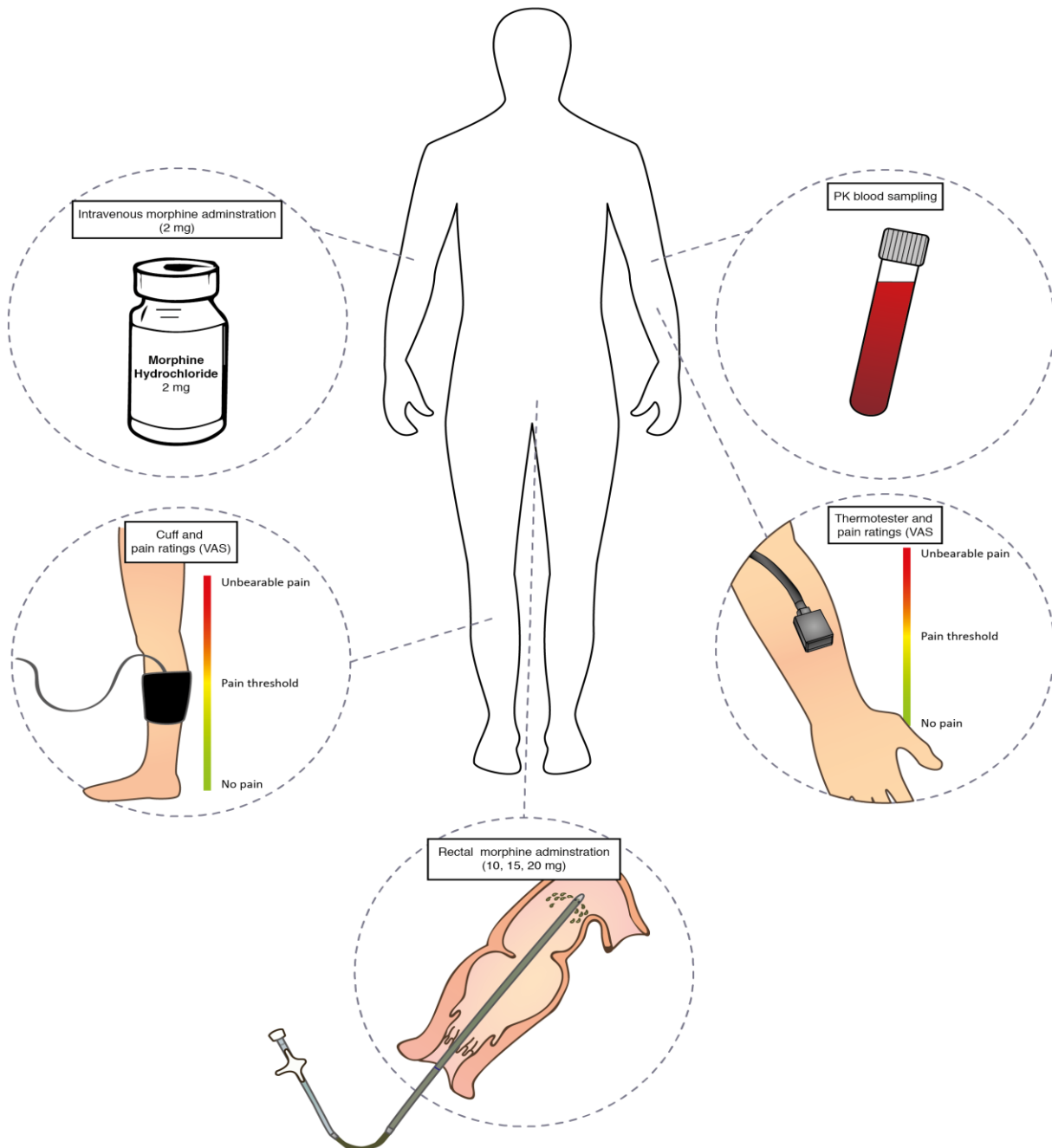


Fig. 4: Experimental setup in study I. Study I was an open label, four-way study with escalating doses of morphine hydrochloride, at the first study visit 2 mg morphine hydrochloride was administered intravenously. The three consecutive study visits 10-, 15-, and 20 mg morphine hydrochloride was administered rectally in ascending order by means of a custom-designed probe. Mechanical muscle stimulation was performed by means of a tourniquet cuff (right leg). Additionally, thermal skin stimulation was performed with a heating thermode. It was applied 10 cm from the elbow joint on the volar surface of the left forearm. The pain sensation was subjectively assessed with a VAS. In addition, PK blood sampling was performed.

3.3.1.5 Registration of adverse effects

Morphine analgesia may be accompanied by several centrally mediated side effects as described in section 1.3.1.2. The adverse effect profile following *rectal* administration of liquid morphine has to some degree been established in patients (59-61). This is best described in cancer patients following long-term treatment (60). The most pronounced symptoms were constipation, drowsiness, nausea, dry mouth and vomiting and 6% of the patients had to discontinue rectal drug administration due to local intolerance. Administration of methylnaltrexone (study II) has been associated with gastrointestinal adverse effects. Altogether, the assessment of tolerability and safety is imperative in the study design. To assess safety and tolerability, the participants were asked to rate the following adverse effects: “nausea”, “dizziness”, “itching”, “sweating”, “sedation” and “lower gastrointestinal complaints” before dosing and 10, 20, 30, 45, 60, 90, 120 and 180 minutes after dosing. Participants were asked to grade the severity of the adverse effects, if any (1=no effects, 2=modest effects, 3=moderate effects and 4=intolerable effects).

3.3.2 Key results

3.3.2.1 Participants

During the period of June 2012 to October 2012 ten males were screened. Their mean age was 25.6 years (range 23-29 years) and mean weight was 77.1 kg (range 63-100 kg). They all met the inclusion criteria and were enrolled in the study. Following rectal morphine administration no leakage was observed in any of the participants.

3.3.2.2 Adverse effects and dose selection

The majority of participants rated the adverse events as being modest. Two participants rated the adverse events as being of moderate nature (one after 10 mg morphine (nausea and “stomach rumble”) and one after 20 mg morphine (dizziness) but none rated them as being intolerable and in addition, no local intolerance or other gastrointestinal complications were observed. The observed adverse effects appeared to be dose independent. As the highest dose (20 mg) was well tolerated, it was decided to further increase the dose in study II, as relative high doses are preferred when analgesic effects are investigated in experimental human pain studies (80). Pharmacokinetic-pharmacodynamic (PK-PD) modelling was used as a simulation tool. Simulations were based on data from mechanical muscle stimulation, as this quantitative sensory test proved to be more sensitive to morphine analgesia than thermal skin stimulation. Mechanical muscle stimulation was thus, to be used in study II (Fig. 5). During the simulations a dose of 30 mg morphine was found to provide an approximately 15% increase (from baseline) in tolerated muscle pressure (106).

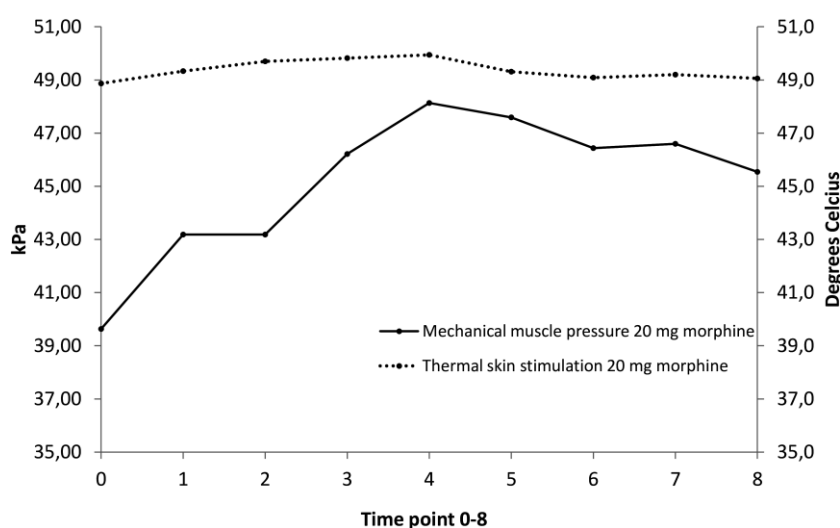


Fig. 5: Solid line represents data from the mechanical muscle stimulation and dotted line represents data from thermal skin stimulation. The pain stimulations were performed at baseline and eight times after dosing according to a matrix. This resulted in the following eight post dose pain stimulation intervals: 2-6 minutes, 8-12 minutes, 14-18 minutes, 21-25 minutes, 30-54 minutes, 60-84 minutes, 90-114 minutes and finally 130-180 minutes.

Data were best described with a two compartment distribution model with one absorption transit compartment for rectal administration and systemic clearance from the central compartment (Fig. 6).

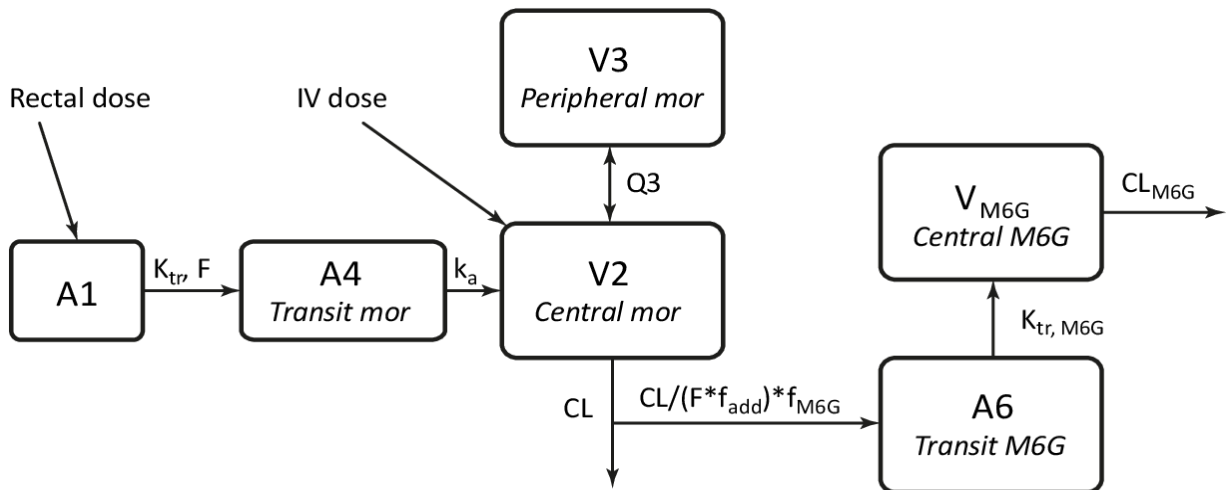


Fig. 6: Final PK model diagram for simultaneous description of serum concentration-time profiles of morphine (mor) and M6G following IV or rectal administration of morphine. A is drug amount in the compartment and numbers denote the compartments (1: dosing; 2 and 3: the central and peripheral compartments of morphine; 4: morphine transit compartment from compartment 1 to 2; 5: central compartment of M6G; 6: transit compartment for M6G between compartment 2 and 5). Q_3 is inter-compartmental clearance. V is volume of distribution. CL_{M6G} is M6G clearance. k_{tr} , $k_{tr, M6G}$ and k_a are first-order rate constants between compartments, F rectal bioavailability and f_{add} is additional fraction of morphine converted to M6G after rectal administration. f_{M6G} is fraction of total systemic morphine clearance converted to M6G.

The mean morphine absorption transit time was 0.6 hours for a typical person (i.e. 70 kg) in the population. Clearance was 78 L/h (relative standard error (RSE) 12 %) and absolute bioavailability was estimated to 24% (RSE 11%). Body weight was suggested to be an important covariate for morphine exposure.

3.4 Study II

3.4.1 Methods

Study II was designed as a randomized, placebo-controlled, double-dummy, double-blinded, four-way cross-over study. Three of four treatment arms were intended for this Ph.D. project and is thus, described in this thesis (II, III). The fourth treatment arm (low dose (0.2 mg) morphine hydrochloride) is to be included in future PK-PD studies.

Fifteen healthy male participants were to complete the study. As in study I, it was anticipated from clinical experience that morphine would have a lag-time of 30 minutes and that the peripheral effect of morphine therefore would be assessable within the first 30 minutes after administration. Thus, to assess the peripheral effects of morphine, pain stimulations were performed as frequent as possible within this “peripheral time frame”. Participants were randomised to three experimental sessions and received two different treatments during each visit: 1) subcutaneous MNTX 12 mg or matching placebo and 2) rectally administered morphine hydrochloride 30 mg or matching placebo giving rise to three different treatment scenarios: 1) placebo+placebo, 2) placebo+morphine hydrochloride 30 mg and 3) MNTX 12 mg mg+morphine hydrochloride 30 mg. According to the PK of MNTX ($T_{\max}=0.5$ hours), MNTX or matching placebo was administered 30 minutes prior to morphine administration in order to obtain maximal effect before morphine dosing. Administration was performed subcutaneously in the right thigh by one of the two research nurses. A washout period of minimum one week was left between the experiments. To reduce the nausea associated with the fast, intravenous administration of 5% glucose was started before initiation of each experiment.

In the remaining document, the placebo+placebo arm will be referred to as “placebo”, placebo+morphine hydrochloride 30 mg arm as “morphine” while the MNTX 12 mg mg+morphine hydrochloride 30 mg arm will be referred to as “MNTX/morphine”. Figure 8 outline the experimental setup.

3.4.1.1 Pain stimulation

The peripheral and central analgesic effect of morphine was assessed by means of mechanical rectal stimulation. In study I, mechanical muscle stimulation proved to be more sensitive to morphine analgesia than thermal skin stimulation and was thus, used in the present study to assess the central analgesic effects of morphine. In addition, transcutaneous electrical stimulation was applied to assess the central analgesic effects.

Visceral stimulation

Experimental visceral pain models have been conducted in different parts of the GI tract (3,97,107). These models enable the investigator to stimulate and thus, activate specific groups of nociceptors in the gut. It is notable that these models are difficult to perform due to localization of the organs and the risk of organ

damage (107). Sensory information from the GI tract is mediated by either unmyelinated C-fibers (70-90%) or thinly myelinated A δ -fibers (108). The proportion of C-fibers increases along the GI tract (from the oral to the anal end) and are localized in mucosa, muscle and serosa. The majority of the visceral nociceptors are polymodal thus, responding to an array of different stimuli (109). In rectal experimental studies, thermal, electrical and mechanical modalities have been applied (97,103,110-112). To assess the effect of morphine and MNTX on visceral pain, mechanical rectal stimulation by means of distension was performed. For this purpose, a rectal probe that allowed for both medicine administration and stimulation was developed. The design was based on the probe design applied in study I and the probe design of a multimodal rectal probe previously developed by our group (112). The probe designed for the present study had a length of 40 cm and had eight lumens. Two of these were intended for inflation and deflation, one for pressure recording, one contained a guide wire to stabilize the probe and essentially four for medicine administration (Fig. 7).

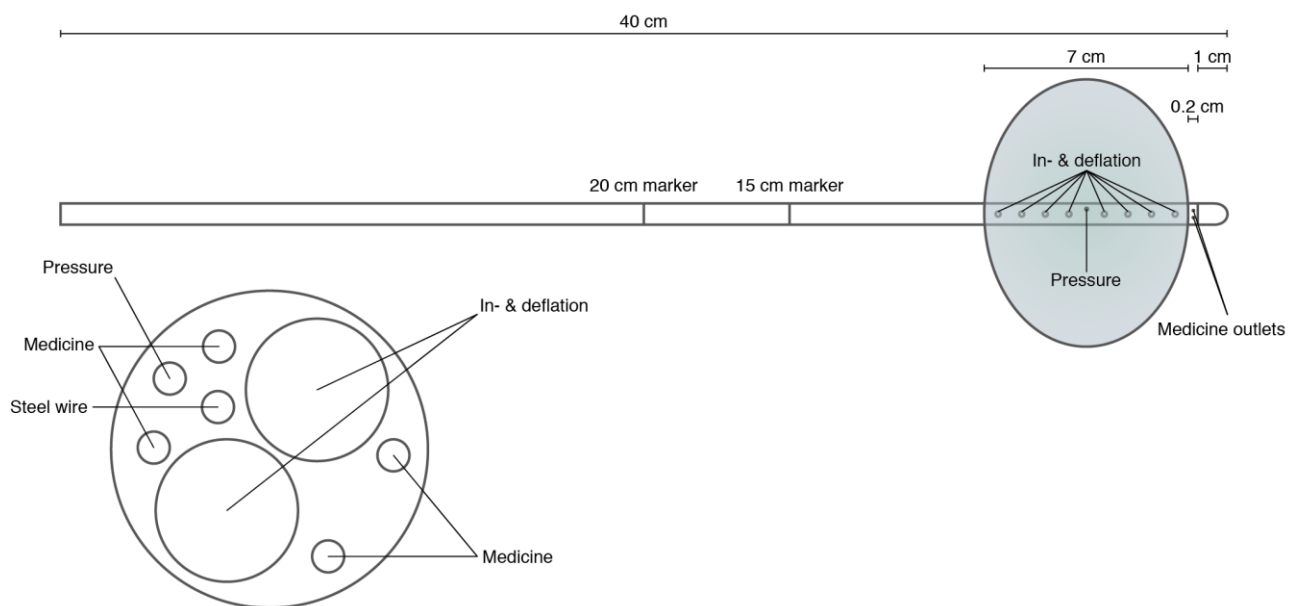


Fig. 7: Schematic drawing of the probe applied in study II including the cross section of the probe (lower left hand side). The probe had a length of 40 cm and eight lumens. Two of these were intended for inflation and deflation of the non-compliant 30 μ m thick polyestherurethane bag which was mounted 1.2 cm proximal to the probe tip. One lumen was intended for pressure recording, one contained a guide wire to stabilize the probe and essentially four for medicine administration. The medicine administration was performed 20 cm from the anal verge (20 cm marker). After drug administration the probe was withdrawn 5 cm and stimulations were performed with the probe placed 15 cm from the anal verge (15 cm marker).

The medicine administration was performed 20 cm from the anal verge. After drug administration the probe was withdrawn 5 cm. Distension was enabled via an inflatable non-compliant 30 µm thick poly-esterurethane bag which was mounted 1.2 cm proximal to the probe tip (Fig. 7). Stimulations were performed with the probe placed 15 cm from the anal verge. The recorded pressure was used to monitor and control the quality of the distension. To precondition the tissue three distensions to the pain threshold (equal to VAS=5) were performed immediately prior to baseline (pre-dose) stimulation (78). Moderate pain (VAS=7) was chosen as the stimulation endpoint. Stimulations were made at baseline and at 1, 7, 15, 30, 60, 120 and 180 minutes after drug administration.

Somatic stimulation

Mechanical muscle stimulation

Mechanical muscle stimulation was performed (as described in section 3.3.1.4) at baseline and 1, 7, 15, 30, 60, 120 and 180 minutes after dosing.

Transcutaneous skin stimulation

Transcutaneous electrical stimuli were applied using a commercially available, hand-held, constant current stimulator (PainMatcher®) (113). A constant current of 15 mA square wave pulses at 10 Hz was sent through the tissue. The pulse duration (i.e. the electrical charge) was increased over time in increments of 4 µsec, from 4 µsec to 396 µsec. Each increment corresponds to one step on an arbitrary scale from 0 to 99.

In the assessment of transcutaneous electrical pain, the pain tolerance threshold (PTT) was applied. The PTT was defined as the maximum intensity of the electrical stimulus the participant was willing to accept in the given situation. The method was chosen on the basis of a previous study of morphine analgesia rendering significant results (114). PainMatcher® has proven to be reliable and reproducible (115,116) and in a practical context, the method could easily be implemented in the study design. Stimulations were made at baseline and at 1, 7, 15, 23, 30, 45, 60, 120 and 180 minutes after drug administration.

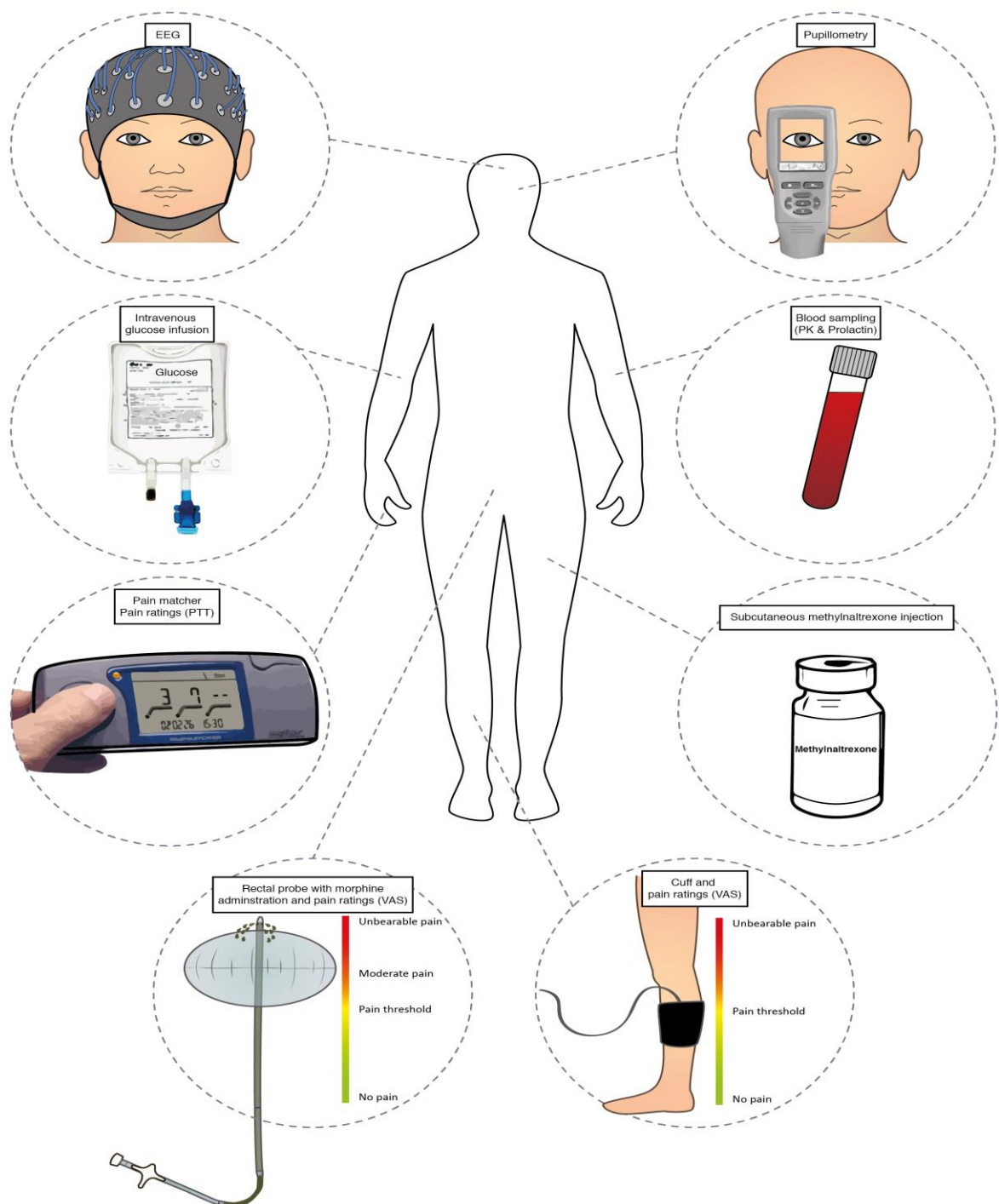


Fig. 8: Experimental setup in study II. 30 mg morphine hydrochloride or matching placebo was administered rectally by means of a custom-designed probe. The same probe allowed for mechanical stimulation of the rectum. The peripherally restricted μ -opioid antagonist, MNTX or matching placebo was administered subcutaneously in the left thigh. Mechanical muscle stimulation was performed by means of a cuff (right leg). Transcutaneous electrical stimulation was performed with Painmatcher® (right hand). The pain sensation was subjectively assessed with a VAS or with PTT (Painmatcher®). Centrally mediated effects were monitored with pupillometry and resting EEG. Additionally, prolactin and PK blood sampling was performed. The different measures were performed at baseline and at different times after drug administration and in the same order: 1. Blood sampling, 2. Pupillometry, 3. Resting EEG, 4. Mechanical rectal stimulation, 5. Mechanical muscle stimulation and 6. Transcutaneous electrical stimulation. To reduce the nausea associated with the fast, intravenous administration of 5% glucose was started before initiation of each experiment.

3.4.1.2 Objective assessments

To objectively assess the impact of opioids in the CNS, pupillometry, prolactin concentration levels and resting EEG was used.

Pupillometry

Pupillometry is a method to measure the pupil diameter. Pupillary constriction can be induced following a sufficient dose of opioid agonists. Opioid induced pupillary constriction is also termed “opioid miosis”. The pupil diameter is regulated through interactions between the sympathetic and parasympathetic nervous systems. Pupillary constriction is mediated through the parasympathetic nervous system (117). However, the mechanisms behind opioid induced miosis remain unclear (118,119). The dominant theory is mainly based on canine studies (120,121). Theories have suggested that opioids directly stimulate the neurons in the pupilloconstrictor nucleus (Edinger-Westphal nucleus) (120,121) while others have suggested more indirect mechanisms (118,122). The pupil diameter is affected by lighting intensity (123). Indeed, Weinhold and Bigelow demonstrated that opioid induced miosis is best detected under moderately dimmed lighting conditions (123). In humans, opioid-induced miosis after administration of different opioids (e.g. morphine, tramadol, remifentanyl and alfentanil) has been demonstrated under ambient lighting condition e.g. (69,124-136) and pupillary response has become a well established objective index of central opioid effect. For this reason, pupillometry was used for assessment of central morphine effect. The recordings were performed under moderately dimmed interior lighting conditions. To be able to manoeuvre in the laboratory during the experiment, the room was lit by 3 pc screens and a dimmed desk lamp. If the lighting conditions changed during the experiment a minimum of two minutes were allowed for dark adaptation before recording. Pupillometry recordings were performed using a commercially available digital infrared hand-held NeuroOptics VIP 200 pupillometer (NeuroOptics, Irvine, CA, USA) (137). Due to the non-invasive and painless character of the method, pupillary recordings were performed at baseline and intensively after dosing (1, 7, 15, 23, 30, 45, 60, 80, 100, 120 and 180 minutes).

Prolactin

It is well recognized that opioids generally stimulates the endocrine system (138) and it has been termed opioid endocrinopathy (45). The opioid induced hormonal effects have been documented in both men and women (138). Various studies, in both animals (138) and humans (139-142), have demonstrated the stimulant effect of morphine on prolactin secretion. In general, acute opioid administration stimulates prolactin secretion, while the effect of chronic opioid administration is less clear (138). It has been suggested that prolactin secretion is mediated via dopaminergic neurons in hypothalamus (143). Therefore, prolactin plasma concentrations were used as a measure of opioid effect in the CNS. Consequently, serial blood sampling (baseline and 1, 7, 15, 23, 30, 45, 60, 120 and 180 minutes after dosing) was performed throughout

the study in order to evaluate the prolactin secretion. Prolactin concentrations were measured using an automated immunofluorometric assay ((KRYPTOR) BRAHMS GmbH, Hennigsdorf, German) (144). The measurement principle is based on Time-Resolved Amplified Cryptate Emission (TRACE™) technology (144,145). In brief, the assay technology is based on non-radiating energy transfer between a donor (an Europium Cryptate labeled antibody) and an acceptor molecule (an XL665 labeled antibody). The donor and acceptor molecules bind to the prolactin (antigen) in the blood sample and form an immunocomplex (antibody-antigen-antibody complex). A nitrogen laser at 337 nm excites the donor which emits a fluorescent signal at 620 nm. The energy is transferred to the acceptor. The acceptor reemits a fluorescent signal at 665 nm which is then measured. The fluorescence is proportional to the antigen (prolactin) concentration (144).

Electroencephalography

EEG is a non-invasive method that reflects the electrical activity in the brain over time. The activity can be recorded as spontaneous EEG or as evoked potentials after an external event such as a painful stimulus. Spontaneous EEG measures the neural activity at rest and was applied in the present study (146). The EEG recording is the sum of excitatory and inhibitory postsynaptic activities (147). These activities are synchronized in a large population of neurons in different brain regions and transmitted to the surface by volume conduction (148). The information contained in the raw EEG signal consists of a multitude of slow and rapid frequency potentials. Despite the chaotic nature, it has been demonstrated that EEG oscillates in distinct frequency bands (quantified by oscillations by second). The assessment of activity in each of the bands can be characterized by spectral analysis. In brief, by applying spectral analysis e.g. by means of traditional fourier transformation or the more advanced continuous wavelet transform, the raw EEG signal is transformed from the time domain into the frequency domain (146,149) (Fig. 9). The transformation results in a power spectrum which can be quantified into standardized frequency bands.

Spontaneous EEG has mainly been used to identify altered cerebral activity following pharmacological intervention with opioids and other centrally acting drugs (150). Collectively, quantitative EEG recordings in the context of drug testing are termed pharmac-EEG (146,151,152). To quantify the effect of morphine on the resting EEG, the signals at vertex (Cz) were analyzed in terms of altered absolute and relative frequency distribution. The frequency distribution was retrieved by a continuous wavelet transform. The absolute distribution was divided in the following frequency bands: delta (0.5 – 3.5 Hz), theta (4 – 7.5 Hz), alpha1 (8 – 10.5 Hz), alpha2 (10.5 – 13.5 Hz), beta1 (14.0 – 18.5 Hz), beta2 (19 – 24.5 Hz), and beta3 (25 – 32 Hz). The absolute distribution was used to calculate the relative distribution for each band as a percentage of the total power in the 0.5-32 Hz range.

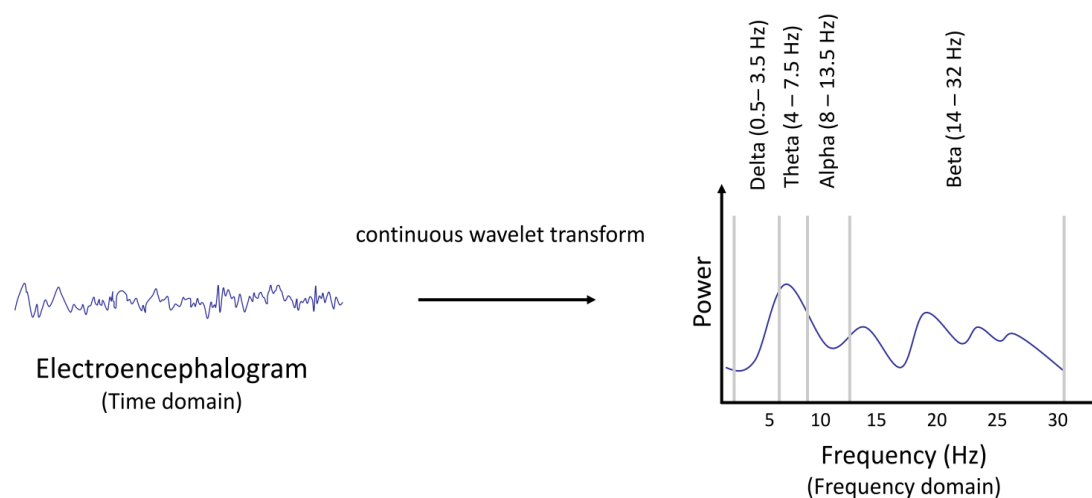


Fig. 9: The parameterization of EEG activity is largely based on spectral analysis, by means of continuous wavelet transform. After digitalization, the EEG signal is transformed from the time domain into the frequency domain. The transform results in a power spectrum that is divided into four bands by frequency. The areas between the gray vertical lines denote the four frequency bands: delta (0.5–3.5 Hz), theta (4–7.5 Hz), alpha (8.0–13.5 Hz), and beta (14.0–32 Hz).

In pharmaco-EEG studies, opioids have generally induced a slowing of the spontaneous EEG seen as an increase of the activity in the delta band (153-156) (reviewed in reference (150)). However, increased activity in higher frequency bands have also been reported (154,157). EEG studies of morphine have produced contradicting results. As an example Löttsch and co-workers demonstrated an increase in the delta band (although the signal did fluctuate over time) and an increase in the alpha-1, beta-1 and beta-2 bands following intravenous morphine administration (157) while another study found no significant alterations in the spontaneous EEG after administration of intramuscular morphine (154).

3.4.1.3 Registration of adverse effects

Registration of adverse effects was performed as described in section 3.3.1.5

3.4.2 Key results

3.4.2.1 Participants

During the period of September 2013 to April 2014 nineteen males were screened. Of these, one did not meet the inclusion criteria due to addictive behaviour in a first degree relative. Thus, a total of eighteen participants enrolled in the study. Three of these participants withdrew their informed consent; one participant due to personal reasons and two participants due to unpleasantness during the study. Consequently, the study was completed by fifteen healthy male participants, mean age 25.5 years (range 20-56) (Fig. 10).

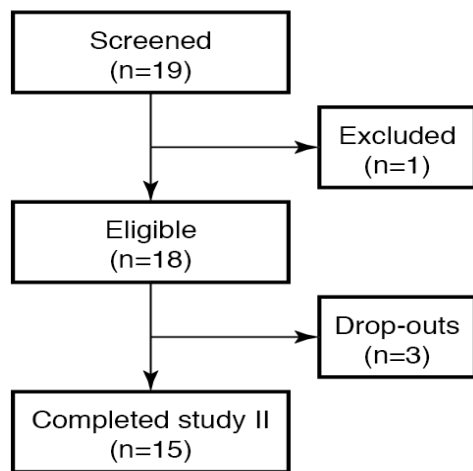


Fig. 10: Participant flow in study II. 19 healthy males were screened. Of these 18 was enrolled in the study. Three participants dropped out after inclusion, thus 15 participants completed the study.

3.4.2.2 Study IIa

No peripheral effect to rectal stimulation was found following morphine administration ($P=0.8$). In contrast, an effect was seen following MNTX/morphine administration ($P<0.001$). The effect was significantly different from placebo seven minutes after morphine administration and continued throughout the experiment. Morphine and MNTX/morphine both had an effect on mechanical muscle pressure ($P<0.001$, both treatments) and on pupil diameter ($P<0.001$, both treatments) compared to placebo. For both mechanical muscle pressure and pupil diameter, the effect occurred 30 minutes after dosing and the effect continued throughout the experiment. However, no change was observed in transcutaneous electrical stimulation following either morphine ($P=0.8$) or MNTX/morphine ($P=0.6$). One or more adverse effects were observed in 11 of 15 participants after administration of morphine, in 10 of 15 participants following MNTX/morphine administration and in 4 of 15 of the participants following placebo administration. No gastrointestinal adverse effects were recorded after MNTX/morphine administration. The majority of

participants rated the adverse effects as being modest. Only two participants rated the adverse effects as being of moderate nature but none rated them as being intolerable (II).

3.4.2.3 Study IIb

In addition to the effect on mechanical muscle pressure and on pupil diameter 30 minutes after dosing, morphine administration led to an increase in prolactin concentration ($P<0.001$) and also exerted an effect on the resting EEG, which was manifested as a decrease in the relative theta (4-7.5 Hz) activity ($P=0.03$). The increase in prolactin concentration became significant 45 minutes after administration (Fig. 11). The change in the relative theta activity was not significant until 120 minutes after dosing and did not correlate to the increase in tolerated muscle pressure ($r=-0.1$, $P=0.43$). On the other hand, the change in pupil diameter was negatively correlated to the change in tolerated muscle pressure ($r=-0.40$, $P<0.001$), whereas the increase in prolactin concentration was positively correlated ($r=0.32$, $P=0.001$) (III).

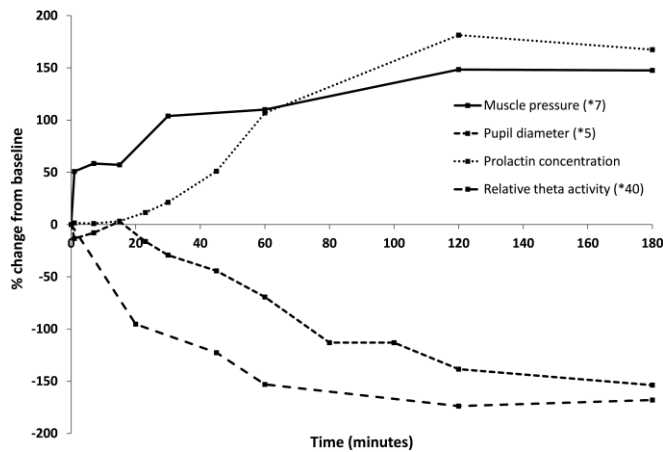


Fig 11: Mean relative change (%) from baseline for tolerated muscle pressure, pupil diameter, prolactin concentration and relative theta activity. The curves represent the difference between morphine and placebo. Muscle pressure data, pupil diameter data and relative theta activity data are magnified to obtain values in the same range as prolactin concentration data (factors are shown in brackets).

4 Discussion

4.1 Study I

In the dose escalation study it was found that liquid morphine administered rectally is safe and well tolerated in healthy male participants in the dosage regime presented. Interestingly, the adverse effects did not appear to be dose dependent. Relative high doses are preferred when analgesic effects are investigated in experimental human pain studies (80) and an oral dose of 30 mg morphine has successfully been administered to healthy participants in various experimental pain studies (103,104,158). In the modelling of morphine serum concentrations after intravenous and rectal administration, the typical value of bioavailability was estimated to 24% for rectally administered morphine, which was found to be dose independent. After oral administration of a 12 mg morphine tablet, an absolute bioavailability of morphine has been reported to $20 \pm 8.3\%$ (159). Accordingly, morphine bioavailability after oral administration appears to be comparable with the bioavailability after rectal administration (I). The low bioavailability suggests that absorption from the upper part of the rectum is subject to extensive first-pass metabolism. Given that the 20 mg dose proved safe and well tolerated; and the population PK model further established that rectal absorption in the upper rectum is subject to extensive first-pass metabolism, it was decided to increase the dose to 30 mg in Study II. Mechanical muscle stimulation proved to be more sensitive to morphine analgesia than thermal skin stimulation. This is in good agreement with a recent population PK-PD modelling study suggesting that mechanical muscle stimulation is more clinically relevant for assessment of morphine pharmacodynamics than is thermal skin stimulation (160).

PK data was best described by a two compartment distribution model with one absorption transit compartment for rectal administration and systemic clearance from the central compartment. The structural model and typical parameter values for morphine's systemic PK are comparable with those previously published using two-compartment models (161,162). Three-compartment models have also been presented for studies with longer sampling duration (163-165). Body weight was incorporated in the final model as allometric scaling of morphine's systemic PK parameters. This finding indicates that clearance rates are perfusion-limited, which is in good agreement with the high extraction ratio evident by a systemic clearance value close to liver blood flow (~ 90 L/h for a 70 kg person) (166).

The present population PK model enabled assessment of variability components for the PK of a drug. For morphine, the absorption process appears to contribute the most to variability between participants and study occasion (IOV for k_a and IIV for k_{tr}). The variability in absorption suggested from the results of our study is in good agreement with the results from the study of Westerling *et al.* (61). It has been suggested that poor mucosal contact contributes to the large variation in both plasma concentration and bioavailability and that enhancement of contact between drug and mucosa will lead to a significant improvement in the inter-individual variation (62). Another way to enhance the bioavailability of morphine may be to adjust the pH of

the solution. According to Moolenaar *et al.* adjustment to alkaline pH significantly increased the extent of morphine absorption (90).

4.2 Study IIa

Neither a central nor a peripheral analgesic effect to rectal stimulation was found following rectal morphine administration. As the method complied with all recommendations, there is no indication that the lack of effect was directly associated with the experimental procedures. There are different possible explanations to why no analgesic effect was observed following morphine administration: 1) It is well known that morphine generally increases rectal resting tone via effect in the enteric nervous system which will result in a less compliant rectum with poorer ability to accommodate volume (16). Thus, increased rectal tone may have distorted our ability to detect an analgesic effect of morphine on the rectal stimulation. 2) Although clinical studies have demonstrated peripheral mediated reduction in pain after local application of opioids, it has primarily been in the presence of inflammation and it has been shown that analgesic effect is less pronounced in non-inflamed tissue (33,34,104,167). The present experimental model was performed in non-inflamed tissue, which may partly explain the lack of a peripheral analgesic effect. 3) The κ -agonists have been suggested to be important modulators of visceral pain and that their effect is primarily mediated in the periphery (36,168). In visceral pain models in animals, κ -agonists have been suggested to be the most effective to attenuate visceral pain (38,39). This notion is further supported by findings from human experimental pain studies where oxycodone proved to be superior to morphine in alleviating visceral pain in both healthy participants and chronic pancreatitis patients (158,169). In contrast to morphine, oxycodone has shown an effect on κ -opioid receptors in animal studies (170-173). This indicates that oxycodone is a partial κ -agonist and it can be speculated that the affinity of κ -receptors may have contributed to the superior analgesic effect in these studies. It is plausible that only κ -agonists exert an effect on the peripheral afferents and thereby are a more effective class of opioids to alleviate visceral pain. 4) Another explanation could be that μ -opioid receptors in the enteric nervous system may be a subgroup of opioid receptors, where binding of an agonist is mainly responsible for motility and not involved in nociception. 5) Although speculative, it could be hypothesized that MNTX administered systemically, will not reach the most peripheral gastrointestinal sensory afferents and thus, it will only block increased motility and not peripherally induced analgesia. This could explain why analgesia was seen as soon as seven minutes after administration of MNTX and morphine. This peripheral morphine analgesia may not be measurable after pure morphine administration as the effect of morphine on motility, will counteract the analgesia. Thus, according to the results from the present study, it cannot be excluded that morphine can exert a peripheral mediated analgesic effect. As aforementioned MNTX/morphine administration led to a significant increase in tolerated volume for rectal stimulation. Interestingly, the increase in tolerated volume became statistically significant only seven minutes after administration when compared to the morphine arm. This was unexpected since MNTX would enable

blockage of peripheral μ -opioid receptors and thus no effects of morphine would be expected within the peripheral timeframe (first 30 minutes). However, as speculated MNTX may not reach the most peripheral gastrointestinal sensory afferents and the effects seen, may be morphine analgesia. It may also be an effect of MNTX. MNTX antagonizes the effect of endogenous as well as exogenous opioids (16). Thus, blocking of opioid receptors in the enteric nervous system by MNTX may lead to a decreased rectal muscle tone (muscle relaxation). Supporting this, results from an animal study showed that naloxone, another opioid antagonist, decreased intraluminal pressure (174). This could explain the increased ability to accommodate more volume and the corresponding pain relief demonstrated in the present study. This suggests that MNTX does not counteract the potential peripheral analgesic effect of morphine on rectal pain but may on the contrary have an indirect analgesic effect on rectal distension. This is also in good agreement with results from a previous study where it was demonstrated that administration of butylscopolamine, a spasmolytic drug, abolished the hypersensitivity in patients with ulcerative colitis (175). This may reflect that opioid antagonists can alleviate gut pain related to distension rather than decreasing any potential local analgesic effect. Thus, the muscle relaxing effect of opioid antagonists may have a higher impact in visceral pain states, than previously assumed.

The dose of 30 mg morphine, estimated by simulations as described under study I, proved sufficient to induce central analgesia after rectal administration (II). The majority of participants experienced adverse effects after either morphine or MNTX/morphine administration. However, the adverse effects were primarily of modest nature which suggests that a 30 mg morphine solution, administered via the rectal route, is safe and well tolerated in healthy male participants. Central analgesia after either morphine or MNTX/morphine treatments was confirmed by attenuation of mechanical muscle pain and a decrease in pupil diameter. In both cases, the central analgesic effect was present 30 minutes after dosing and sustained throughout the study. The morphine induced miosis observed in the present study is in good agreement with previous studies of opioid induced miosis (124,126,129,130,133,135). After morphine administration, a maximum effect in tolerated muscle pain occurred after 60 minutes, where a 22% change from baseline was observed. This change is considerable higher than the initial target response of 15 %. The onset of central analgesia in the present study corresponds well with the onset of central analgesia after oral administration. On the other hand, none of the morphine treatments increased the sensory response to transcutaneous electrical stimulation, when compared to placebo. This is inconsistent with results from a previous study of Ravn *et al.* (114) where an increase in pain tolerance threshold following morphine administration were reported, when using Painmatcher®. However, differences in the study designs may account for the discrepancy. While a single rectal dose of 30 mg was administered in the present study, Ravn *et al.* administered low- and high dose morphine (10 mg and 20 mg, respectively) as intravenous infusions over a 210 minute period. Considering the first-pass metabolism, the administered dose may have been too low to increase the sensory response to transcutaneous electrical stimulation.

4.3 Study IIb

In addition to the decreased pupil diameter and increased muscle pain tolerance, morphine led to a significant increase in prolactin plasma concentration and a decrease in EEG oscillations in the relative theta band (III). Morphine had an effect on prolactin concentration 45 minutes after dosing and thus, shortly after subjective analgesia and pupillary response occurred. The time course of the increased prolactin concentrations observed after morphine administration corresponded reasonably well with the time course for central analgesia and results are consistent with results from previous studies on the effects of morphine on the endocrine system (139,141) (III).

In the present study both absolute and relative EEG activity was assessed. However, while no alterations were observed in any of the bands for the absolute distribution, a decrease in relative theta activity was seen. The decrease in relative theta activity, however, was not significant until 120 minutes after dosing and did not correlate to the mechanical muscle pain. Equivalent to the findings in the present study, decreased relative theta band activity has also been reported in a study on the frequency distribution after remifentanyl infusion in healthy male participants (176). This could indicate that relative theta band oscillations, which are associated with the cortico-thalamic networks (10), are overruled by the electrical activity in other bands after opioid administration. The late onset of EEG alterations, 120 minutes after dosing, may be a result of relative short EEG segments (45 seconds) compared to traditional recordings of several minutes. In pharmaco-EEG studies, effects of opioids have traditionally been assessed by means of frequency distribution (150). In study II, the assessment of the frequency distribution was based on a single channel at the vertex which is a crude quantification of brain activity. Other analysis methods such as source localisation or network analysis may have been applied to gain increased insight to the cortical mechanism. However, due to the complexity in the methodologies they are not considered simple objective measures.

Both prolactin concentration and pupil diameter showed similar temporal development and both measures had good dynamic ranges. Additionally, both objective measures correlated to the subjective measure and thus, proved to be sensitive objective bed-side measures of morphine effect. However, from a practical and economical perspective, pupillometry is more feasible and is therefore recommended in experimental pain studies where simple objective assessments of the morphine effect are needed.

4.4 Methodological considerations

4.4.1 Study population

In both study I and II healthy male participants aged 18-65 years were recruited. Females were disregarded due to logistical challenges and to save time. The nature of pain research and in particular research performed in the GI tract, will attract some individuals while fending off others. In addition, it tends to be less complicated to recruit students as they are more flexible time wise. By nature, students are generally in

their twenties. Only one out of 25 randomized participants (both studies) was middle aged; the remaining participants ranged between 18-29 years resulting in a homogenous group of healthy young male participants. Altogether, these methodological factors can lead to selection bias. Selection bias is inevitable in experimental pain research, particularly with respect to ethical considerations. Thus, the findings may not be generalized to the Danish population. However, the present Ph.D. project was conducted to increase knowledge about specific mechanisms and not to generalize to a certain population. On the other hand, the homogenous study population may have enhanced the internal validity.

4.4.2 Rectal drug administration

A high variability in absorption was seen after rectal administration in study I, which is consistent with previous findings (61,90). To facilitate a complete drug administration in study I and II, the rectal probe was perfused with 1 or 2 mL isotonic saline, respectively. Following administration no leakage was observed implying that the full dose was sufficiently administered. Body position and movement as well as individual rectal secretion are factors that may contribute to variability in absorption, as seen in the study I. In study I the participants were asked to calmly sit upright immediately after drug administration. The movement and the influence of gravity may have contributed to spreading of morphine in the rectum, although it seems unlikely with a volume of only 6 ml (93). Nevertheless, in an attempt to improve the variability in absorption, the participants remained in the left lateral position after drug administration in study II. However, as population PK analysis for study II has not been performed up till now, the impact of this is yet to be seen. Variation in absorption caused by dysfunctional motility and adherence to faeces was considered negligible due to the fact that 1) the participants had normal bowel movements and 2) before initiation of each experiment the participants were given a bowel-cleansing enema.

4.4.3 The experimental procedure

Study II was double-blinded and thus, the investigators did not know the actual treatment allocation. However, when morphine or other opioids are administered in experimental pain studies, blinding can be difficult as CNS mediated adverse effects can be easily recognized in some participants and this may expose the treatment allocation both to the investigators and to the participants. This may have induced behavioral modifications in the participants in order to mirror the behaviour that they thought the investigator wished to see. On the other hand, the participants were informed that the long fast in combination with the lengthy and comprehensive experimental procedure was likely to cause tiredness and maybe even dizziness, thus cause effects similar to those experienced after administration of morphine. It is noteworthy that not all participants experienced adverse effects during the experimental session but in the proportion of participants who did, it may have induced bias the pain assessment. The effect of opioids is normally assessed by the subjective response to analgesia which may be confounded by several factors: 1) Repetitive stimuli can induce

sensitization (increased response) or habituation (decreased or ceased response) and have the potential to distort the pain assessment (177). In study II, habituation to muscle pressure stimuli was observed. However, as the study was placebo-controlled, the induced habituation did not affect the results on drug effects; 2) Pain is a subjective experience, which is influenced by various factors including anxiety and fear. Pain in patients is frequently associated with emotional distress, which can bias the pain perception. However, experimental pain assessment in healthy participants, as the case in study I and II, is less biased. 3) Subjective pain assessment requires full attention from the participant and opioid induced sedation and nausea could potentially influence the pain rating. In healthy participants however, it has been suggested that experimental pain may increase arousal levels and counteract the sedative effect accordingly (178). This notion was supported by results from an experimental pain study reporting an increased finger tapping frequency after both placebo and opioid administration suggesting an increased alertness (104). Regardless of these arguments it cannot be fully avoided that the subjective assessments of pain can be confounded.

4.4.4 Study design

Choice of study design was based on what would be best suited for addressing the objectives of this Ph.D. project. The project should be achievable within the three years available for a Ph.D. project, as well as adequately answering the research questions of this thesis with respect to validity and practicality. Experimental rectal pain can be evoked with different modalities (80). In our group thermal, electrical and mechanical methods have been established (111). However, due to the comprehensive testing regime in study II it was not possible to include more than one pain measure. Retrospectively, inclusion of more than one visceral pain measure may have enabled us to demonstrate peripheral morphine effects and thus, may have improved the study design. Along the same line, inclusion of a MNTX/placebo arm would undoubtedly have enabled a more precise assessment the effects of MNTX.

5 Conclusions

The three studied doses proved to be safe and well tolerated. Morphine was absorbed with a bioavailability which corresponds well with the bioavailability seen after oral administration. Mechanical muscle stimulation proved to be more sensitive to morphine analgesia compared to thermal skin stimulation and a morphine dose of 30 mg was estimated to be optimal for the proceeding study. Thus, the findings enabled dose selection and quantitative sensory test optimization. In addition, a population PK model of liquid rectal morphine and M6G was developed.

No peripheral analgesic effect of morphine was found. On the other hand, MNTX may have exerted a local effect on rectal distensions and thereby appeared to improve analgesia. Methodological shortcomings in the experimental study design may have contributed to the lack of peripheral morphine analgesia and thus, a peripheral effect of morphine on rectal pain cannot be excluded. A dose of 30 mg rectal morphine did however prove to be adequate to induce centrally mediated analgesia. This was manifested as an increase in tolerance to mechanical muscle pressure, a decrease in pupil diameter and an increase in prolactin concentration. An effect was also seen on resting EEG. Although, morphine did induce an effect on resting EEG, it was not considered a sensitive measure of morphine as the effect was considerable delayed compared to the analgesic effect observed on mechanically evoked muscle pain. Thus, only pupil diameter and prolactin concentration proved to be sensitive objective measures of central morphine analgesia. From a practical and economical perspective, pupil diameter is recommended as an objective measure of morphine induced central activation when a simple measurement is needed.

6 Future perspectives

It was not possible to demonstrate peripheral analgesic effects of morphine in study II. Methodological shortcomings in the experimental study design (e.g. increased tone and lack of inflammation) may have contributed to the lack of peripheral morphine effects. As resting tone is affected by morphine, it would be rational to investigate the peripheral effect of morphine on a rectal stimulation modality, where the physiology is not influenced by morphine. Such a method could be heat or electrical stimulation. Models where the cross-sectional area is computed should be included to address the morphine effects on tone and stiffness of the rectal wall. Additionally, as there is substantial evidence that peripheral opioid analgesia is enhanced in the presence of inflammation it would be equally rational to investigate the peripheral effect of morphine in an inflammatory visceral model. In previous preclinical and clinical studies, κ -agonists have proven to attenuate visceral pain and thus, it would be rational to compare the effects of morphine to those of a κ -agonist. Oxycodone is a widely used opioid in the clinic. Although oxycodone is only a partial κ -agonist, previous human experimental pain studies have demonstrated that oxycodone has a better analgesic profile in visceral pain after systemic administration. In future studies, the effects of oxycodone or novel κ -agonists should be compared to those of morphine. Considering the findings from study II, the effects of antagonists on the GI tract should be further addressed. To more precisely assess the contribution of MNTX, a pure MNTX arm should be included in a future study.

The PK samples attained in study II will, together with the PK and PD findings from both studies, be included in future PK/PD modelling. In addition to this, the forth treatment arm from study II will be included in the modelling. The purpose is to quantitatively link the objective markers of central opioid receptor activation with morphine plasma concentration and analgesia.

Table 1: Rectally administered liquid morphine in adults

Authors	Drug, dose, formulation & administration	Design	Bioavailability	Comments
Pannuti et al. 1982 (60)	Morphine hydrochloride Rectal dose: 10 mg administered every 4 hrs by a insulin-type syringe w/rubber tube diluted in distilled H ₂ O to a concentration of 5 mg/ml	N=37 cancer patients, 18-75 yrs	NA	Enema twice a week if patients did not empty their bowels spontaneously. Two of 37 patients had to discontinue treatment due to local intolerance.
Westerling et al. 1982 (61)	Morphine chloride 10 mg/ml Rectal dose: 0,3 mg/kg (~15-27.9 mg) Administration w/special applicator, not described in details.	N=21 patients undergoing surgery (F), 32-72 yrs Weight: 58-93 kg Rectal and intramuscular administration	31 % (12%-61%) Determined in a subset of patients (n=6) who received an intramuscular morphine injection.	Marked interindividual variation in plasma concentration. No information regarding bowel cleaning prior to the study. Adsorption to faeces may have contributed to large to interindividual variation.
Moolenaar et al. 1985 (90)	Morphine hydrochloride Rectal dose: 10 mg dissolved in 1) 5 ml citrate phosphate buffer (pH=4.5) and 2) 5 ml of solution adjusted to pH 7.4 5 ml syringe w/ a plastic applicator tube	N=7 (M+F), healthy participants, 21-26 yrs Rectal and oral administration (cross-over study)	NA (only AUC given)	Increase in absorption rate and bioavailability when adjusting pH to 7.4.
De Conno et al. 1995 (59)	Morphine hydrochloride Rectal dose: 10 mg (for 2 days) Administration with a syringe	N=34 cancer patients Rectal and oral administration (cross-over study)	NA	Liquid morphine is well absorbed Efficacy and safety comparable with oral morphine. Pain relief achieved faster and maintained better after rectal administration.

AUC: Area under the curve

%: Percent

W/: With

F: Female

M: Male

Yrs: Years

Hrs: Hours

7 Reference list

- (1) Mersky H, Bogduk N editors. Classification of chronic pain: descriptions of chronic pain syndromes and definitions of pain terms. second ed. Seattle: IASP Press; 1994.
- (2) Melzack, R, Casey, KL. Sensory, motivational and central control determinants of pain: a new conceptual model. In: Kenshalo D, editor. The Skin Senses Springfield, Illinois, USA: CC Thomas; 1968. p. 423-439.
- (3) Drewes AM, Gregersen H, Arendt-Nielsen L. Experimental pain in gastroenterology: a reappraisal of human studies. Scand J Gastroenterol 2003 Nov;38(11):1115-1130.
- (4) Cervero F, Laird JM. Visceral pain. Lancet 1999 Jun 19;353(9170):2145-2148.
- (5) Langley P, Muller-Schwefe G, Nicolaou A, Liedgens H, Pergolizzi J, Varrassi G. The impact of pain on labor force participation, absenteeism and presenteeism in the European Union. J Med Econ 2010;13(4):662-672.
- (6) Langley P, Muller-Schwefe G, Nicolaou A, Liedgens H, Pergolizzi J, Varrassi G. The societal impact of pain in the European Union: health-related quality of life and healthcare resource utilization. J Med Econ 2010;13(3):571-581.
- (7) Giesecke T, Gracely RH, Williams DA, Geisser ME, Petzke FW, Clauw DJ. The relationship between depression, clinical pain, and experimental pain in a chronic pain cohort. Arthritis Rheum 2005 May;52(5):1577-1584.
- (8) Breivik H, Collett B, Ventafridda V, Cohen R, Gallacher D. Survey of chronic pain in Europe: prevalence, impact on daily life, and treatment. Eur J Pain 2006 May;10(4):287-333.
- (9) Merchand S. Applied Pain Neurophysiology. In: Beaulieu P, Lussier D, Porreca F, Dickenson A, editors. Pharmacology of Pain Seattle: IASP Press; 2010. p. 3-26.
- (10) Todd AJ. Neuronal circuitry for pain processing in the dorsal horn. Nat Rev Neurosci 2010 Dec;11(12):823-836.
- (11) Basbaum AI, Bautista DM, Scherrer G, Julius D. Cellular and molecular mechanisms of pain. Cell 2009 Oct 16;139(2):267-284.
- (12) D'Mello R, Dickenson AH. Spinal cord mechanisms of pain. Br J Anaesth 2008 Jul;101(1):8-16.
- (13) Iannetti GD, Mouraux A. From the neuromatrix to the pain matrix (and back). Exp Brain Res 2010 Aug;205(1):1-12.
- (14) Heinricher MM, Tavares I, Leith JL, Lumb BM. Descending control of nociception: Specificity, recruitment and plasticity. Brain Res Rev 2009 Apr;60(1):214-225.
- (15) Poulsen JL, Brock C, Olesen AE, Nilsson M, Drewes AM. Clinical potential of naloxegol in the management of opioid-induced bowel dysfunction. Clin Exp Gastroenterol 2014 Sep 19;7:345-358.
- (16) Brock C, Olesen SS, Olesen AE, Frokjaer JB, Andresen T, Drewes AM. Opioid-induced bowel dysfunction: pathophysiology and management. Drugs 2012 Oct 1;72(14):1847-1865.
- (17) World Health Organization. Cancer Pain Relief with a Guide to Opioid Availability. 2nd ed. Geneva: World Health Organization; 1996.
- (18) Trescot AM, Datta S, Lee M, Hansen H. Opioid pharmacology. Pain Physician 2008 Mar;11(2 Suppl):S133-53.

- (19) Fioravanti B, Vanderah TW. The ORL-1 receptor system: are there opportunities for antagonists in pain therapy? *Curr Top Med Chem* 2008;8(16):1442-1451.
- (20) Stein C, Lang LJ. Peripheral mechanisms of opioid analgesia. *Curr Opin Pharmacol* 2009 Feb;9(1):3-8.
- (21) Stein C, Schafer M, Machelska H. Attacking pain at its source: new perspectives on opioids. *Nat Med* 2003 Aug;9(8):1003-1008.
- (22) Mousa SA, Straub RH, Schafer M, Stein C. Beta-endorphin, Met-enkephalin and corresponding opioid receptors within synovium of patients with joint trauma, osteoarthritis and rheumatoid arthritis. *Ann Rheum Dis* 2007 Jul;66(7):871-879.
- (23) Kalso E, Tramer MR, Carroll D, McQuay HJ, Moore RA. Pain relief from intra-articular morphine after knee surgery: a qualitative systematic review. *Pain* 1997 Jun;71(2):127-134.
- (24) Likar R, Schafer M, Paulak F, Sittl R, Pipam W, Schalk H, et al. Intraarticular morphine analgesia in chronic pain patients with osteoarthritis. *Anesth Analg* 1997 Jun;84(6):1313-1317.
- (25) Stein C, Comisel K, Haimel E, Yassouridis A, Lehrberger K, Herz A, et al. Analgesic effect of intraarticular morphine after arthroscopic knee surgery. *N Engl J Med* 1991 Oct 17;325(16):1123-1126.
- (26) Stein A, Yassouridis A, Szopko C, Helmke K, Stein C. Intraarticular morphine versus dexamethasone in chronic arthritis. *Pain* 1999 Dec;83(3):525-532.
- (27) Likar R, Koppert W, Blatnig H, Chiari F, Sittl R, Stein C, et al. Efficacy of peripheral morphine analgesia in inflamed, non-inflamed and perineural tissue of dental surgery patients. *J Pain Symptom Manage* 2001 Apr;21(4):330-337.
- (28) Likar R, Sittl R, Gragger K, Pipam W, Blatnig H, Breschan C, et al. Peripheral morphine analgesia in dental surgery. *Pain* 1998 May;76(1-2):145-150.
- (29) Kalso E, Smith L, McQuay HJ, Andrew Moore R. No pain, no gain: clinical excellence and scientific rigour--lessons learned from IA morphine. *Pain* 2002 Aug;98(3):269-275.
- (30) Wenk HN, Brederson JD, Honda CN. Morphine directly inhibits nociceptors in inflamed skin. *J Neurophysiol* 2006 Apr;95(4):2083-2097.
- (31) Machelska H, Stein C. Immune mechanisms in pain control. *Anesth Analg* 2002 Oct;95(4):1002-8, table of contents.
- (32) Stein C, Zollner C. Opioids and sensory nerves. *Handb Exp Pharmacol* 2009;(194):495-518. doi(194):495-518.
- (33) Antonijevec I, Mousa SA, Schafer M, Stein C. Perineurial defect and peripheral opioid analgesia in inflammation. *J Neurosci* 1995 Jan;15(1 Pt 1):165-172.
- (34) Rittner HL, Hackel D, Yamdeu RS, Mousa SA, Stein C, Schafer M, et al. Antinociception by neutrophil-derived opioid peptides in noninflamed tissue--role of hypertonicity and the perineurium. *Brain Behav Immun* 2009 May;23(4):548-557.
- (35) Philippe D, Chakass D, Thuru X, Zerbib P, Tsicopoulos A, Geboes K, et al. Mu opioid receptor expression is increased in inflammatory bowel diseases: implications for homeostatic intestinal inflammation. *Gut* 2006 Jun;55(6):815-823.
- (36) De Schepper HU, Cremonini F, Park MI, Camilleri M. Opioids and the gut: pharmacology and current clinical experience. *Neurogastroenterol Motil* 2004 Aug;16(4):383-394.

- (37) Sternini C, Patierno S, Selmer IS, Kirchgessner A. The opioid system in the gastrointestinal tract. *Neurogastroenterol Motil* 2004 Oct;16 Suppl 2:3-16.
- (38) Sengupta JN, Snider A, Su X, Gebhart GF. Effects of kappa opioids in the inflamed rat colon. *Pain* 1999 Feb;79(2-3):175-185.
- (39) Sengupta JN, Su X, Gebhart GF. Kappa, but not mu or delta, opioids attenuate responses to distention of afferent fibers innervating the rat colon. *Gastroenterology* 1996 Oct;111(4):968-980.
- (40) Su X, Sengupta JN, Gebhart GF. Effects of kappa opioid receptor-selective agonists on responses of pelvic nerve afferents to noxious colorectal distension. *J Neurophysiol* 1997 Aug;78(2):1003-1012.
- (41) Burton MB, Gebhart GF. Effects of kappa-opioid receptor agonists on responses to colorectal distension in rats with and without acute colonic inflammation. *J Pharmacol Exp Ther* 1998 May;285(2):707-715.
- (42) Sandner-Kiesling A, Pan HL, Chen SR, James RL, DeHaven-Hudkins DL, Dewan DM, et al. Effect of kappa opioid agonists on visceral nociception induced by uterine cervical distension in rats. *Pain* 2002 Mar;96(1-2):13-22.
- (43) Eisenach JC, Carpenter R, Curry R. Analgesia from a peripherally active kappa-opioid receptor agonist in patients with chronic pancreatitis. *Pain* 2003 Jan;101(1-2):89-95.
- (44) Arendt-Nielsen L, Olesen AE, Staahl C, Menzaghi F, Kell S, Wong GY, et al. Analgesic efficacy of peripheral kappa-opioid receptor agonist CR665 compared to oxycodone in a multi-modal, multi-tissue experimental human pain model: selective effect on visceral pain. *Anesthesiology* 2009 Sep;111(3):616-624.
- (45) Benyamin R, Trescot AM, Datta S, Buenaventura R, Adlaka R, Sehgal N, et al. Opioid complications and side effects. *Pain Physician* 2008 Mar;11(2 Suppl):S105-20.
- (46) Rosenblum A, Marsch LA, Joseph H, Portenoy RK. Opioids and the treatment of chronic pain: controversies, current status, and future directions. *Exp Clin Psychopharmacol* 2008 Oct;16(5):405-416.
- (47) Wachholtz A, Gonzalez G, Boyer E, Naqvi ZN, Rosenbaum C, Ziedonis D. Intersection of chronic pain treatment and opioid analgesic misuse: causes, treatments, and policy strategies. *Subst Abuse Rehabil* 2011 Aug 18;2:145-162.
- (48) Nicholson B. Responsible prescribing of opioids for the management of chronic pain. *Drugs* 2003;63(1):17-32.
- (49) Kumar L, Barker C, Emmanuel A. Opioid-induced constipation: pathophysiology, clinical consequences, and management. *Gastroenterol Res Pract* 2014;2014:141737.
- (50) Bannwarth B. Risk-benefit assessment of opioids in chronic noncancer pain. *Drug Saf* 1999 Oct;21(4):283-296.
- (51) Drewes AM, Jensen RD, Nielsen LM, Droney J, Christrup LL, Arendt-Nielsen L, et al. Differences between opioids: pharmacological, experimental, clinical and economical perspectives. *Br J Clin Pharmacol* 2013 Jan;75(1):60-78.
- (52) Donnelly S, Davis MP, Walsh D, Naughton M, World Health Organization. Morphine in cancer pain management: a practical guide. *Support Care Cancer* 2002 Jan;10(1):13-35.
- (53) Lugo RA, Kern SE. Clinical pharmacokinetics of morphine. *J Pain Palliat Care Pharmacother* 2002;16(4):5-18.
- (54) Radbruch L, Trottenberg P, Elsner F, Kaasa S, Caraceni A. Systematic review of the role of alternative application routes for opioid treatment for moderate to severe cancer pain: an EPCRC opioid guidelines project. *Palliat Med* 2011 Jul;25(5):578-596.

- (55) Bruera E, Fainsinger R, Spachynski K, Babul N, Harsanyi Z, Darke AC. Clinical efficacy and safety of a novel controlled-release morphine suppository and subcutaneous morphine in cancer pain: a randomized evaluation. *J Clin Oncol* 1995 Jun;13(6):1520-1527.
- (56) Babul N, Provencher L, Laberge F, Harsanyi Z, Moulin D. Comparative efficacy and safety of controlled-release morphine suppositories and tablets in cancer pain. *J Clin Pharmacol* 1998 Jan;38(1):74-81.
- (57) Babul N, Darke AC, Anslow JA, Krishnamurthy TN. Pharmacokinetics of two novel rectal controlled-release morphine formulations. *J Pain Symptom Manage* 1992 Oct;7(7):400-405.
- (58) Campbell WI. Rectal controlled-release morphine: plasma levels of morphine and its metabolites following the rectal administration of MST Continus 100 mg. *J Clin Pharm Ther* 1996 Apr;21(2):65-71.
- (59) De Conno F, Ripamonti C, Saita L, MacEachern T, Hanson J, Bruera E. Role of rectal route in treating cancer pain: a randomized crossover clinical trial of oral versus rectal morphine administration in opioid-naïve cancer patients with pain. *J Clin Oncol* 1995 Apr;13(4):1004-1008.
- (60) Pannuti F, Rossi AP, Iafelice G, Marraro D, Camera P, Cricca A, et al. Control of chronic pain in very advanced cancer patients with morphine hydrochloride administered by oral, rectal and sublingual route. Clinical report and preliminary results on morphine pharmacokinetics. *Pharmacol Res Commun* 1982 Apr;14(4):369-380.
- (61) Westerling D, Lindahl S, Andersson KE, Andersson A. Absorption and bioavailability of rectally administered morphine in women. *Eur J Clin Pharmacol* 1982;23(1):59-64.
- (62) Westerling D, Andersson KE. Rectal administration of morphine hydrogel: absorption and bioavailability in women. *Acta Anaesthesiol Scand* 1984 Oct;28(5):540-543.
- (63) Wilkinson TJ, Robinson BA, Begg EJ, Duffull SB, Ravenscroft PJ, Schneider JJ. Pharmacokinetics and efficacy of rectal versus oral sustained-release morphine in cancer patients. *Cancer Chemother Pharmacol* 1992;31(3):251-254.
- (64) Jonsson T, Christensen CB, Jordening H, Frolund C. The bioavailability of rectally administered morphine. *Pharmacol Toxicol* 1988 Apr;62(4):203-205.
- (65) Du X, Skopp G, Aderjan R. The influence of the route of administration: a comparative study at steady state of oral sustained release morphine and morphine sulfate suppositories. *Ther Drug Monit* 1999 Apr;21(2):208-214.
- (66) Gourlay GK. Sustained relief of chronic pain. Pharmacokinetics of sustained release morphine. *Clin Pharmacokinet* 1998 Sep;35(3):173-190.
- (67) Kilpatrick GJ, Smith TW. Morphine-6-glucuronide: actions and mechanisms. *Med Res Rev* 2005 Sep;25(5):521-544.
- (68) Christrup LL. Morphine metabolites. *Acta Anaesthesiol Scand* 1997 Jan;41(1 Pt 2):116-122.
- (69) Skarke C, Darimont J, Schmidt H, Geisslinger G, Lotsch J. Analgesic effects of morphine and morphine-6-glucuronide in a transcutaneous electrical pain model in healthy volunteers. *Clin Pharmacol Ther* 2003 Jan;73(1):107-121.
- (70) Grace D, Fee JP. A comparison of intrathecal morphine-6-glucuronide and intrathecal morphine sulfate as analgesics for total hip replacement. *Anesth Analg* 1996 Nov;83(5):1055-1059.
- (71) Hanna MH, Peat SJ, Woodham M, Knibb A, Fung C. Analgesic efficacy and CSF pharmacokinetics of intrathecal morphine-6-glucuronide: comparison with morphine. *Br J Anaesth* 1990 May;64(5):547-550.

- (72) Cherny NI. Opioid analgesics: comparative features and prescribing guidelines. *Drugs* 1996 May;51(5):713-737.
- (73) Thomas J, Karver S, Cooney GA, Chamberlain BH, Watt CK, Slatkin NE, et al. Methylnaltrexone for opioid-induced constipation in advanced illness. *N Engl J Med* 2008 May 29;358(22):2332-2343.
- (74) Yuan CS, Doshan H, Charney MR, O'connor M, Karrison T, Maleckar SA, et al. Tolerability, gut effects, and pharmacokinetics of methylnaltrexone following repeated intravenous administration in humans. *J Clin Pharmacol* 2005 May;45(5):538-546.
- (75) Neefjes EC, van der Vorst MJ, Boddaert MS, Zuurmond WW, van der Vliet HJ, Beeker A, et al. Clinical evaluation of the efficacy of methylnaltrexone in resolving constipation induced by different opioid subtypes combined with laboratory analysis of immunomodulatory and antiangiogenic effects of methylnaltrexone. *BMC Palliat Care* 2014 Aug 20;13:42-684X-13-42. eCollection 2014.
- (76) European Medicines Agency. ANNEX I SUMMARY OF PRODUCT CHARACTERISTICS. Available at:http://ec.europa.eu/health/documents/community-register/2008/2008070246452/anx_46452_en.pdf. Accessed 10/10, 2014.
- (77) Mao J. Translational pain research: achievements and challenges. *J Pain* 2009 Oct;10(10):1001-1011.
- (78) Drewes AM, Gregersen H, Arendt-Nielsen L. Experimental pain in gastroenterology: a reappraisal of human studies. *Scand J Gastroenterol* 2003 Nov;38(11):1115-1130.
- (79) Handwerker HO, Kobal G. Psychophysiology of experimentally induced pain. *Physiol Rev* 1993 Jul;73(3):639-671.
- (80) Olesen AE, Andresen T, Staahl C, Drewes AM. Human experimental pain models for assessing the therapeutic efficacy of analgesic drugs. *Pharmacol Rev* 2012 Jul;64(3):722-779.
- (81) Staahl C, Olesen AE, Andresen T, Arendt-Nielsen L, Drewes AM. Assessing analgesic actions of opioids by experimental pain models in healthy volunteers - an updated review. *Br J Clin Pharmacol* 2009 Aug;68(2):149-168.
- (82) Unruh AM. Gender variations in clinical pain experience. *Pain* 1996 May-Jun;65(2-3):123-167.
- (83) Hurley RW, Adams MC. Sex, gender, and pain: an overview of a complex field. *Anesth Analg* 2008 Jul;107(1):309-317.
- (84) Greenspan JD, Craft RM, LeResche L, Arendt-Nielsen L, Berkley KJ, Fillingim RB, et al. Studying sex and gender differences in pain and analgesia: a consensus report. *Pain* 2007 Nov;132 Suppl 1:S26-45.
- (85) Riley JL,3rd, Robinson ME, Wise EA, Myers CD, Fillingim RB. Sex differences in the perception of noxious experimental stimuli: a meta-analysis. *Pain* 1998 Feb;74(2-3):181-187.
- (86) Fillingim RB, King CD, Ribeiro-Dasilva MC, Rahim-Williams B, Riley JL,3rd. Sex, gender, and pain: a review of recent clinical and experimental findings. *J Pain* 2009 May;10(5):447-485.
- (87) Berkley KJ. Sex differences in pain. *Behav Brain Sci* 1997 Sep;20(3):371-80; discussion 435-513.
- (88) Wiesenfeld-Hallin Z. Sex differences in pain perception. *Gend Med* 2005 Sep;2(3):137-145.
- (89) Dixon JR,Jr. The International Conference on Harmonization Good Clinical Practice guideline. *Qual Assur* 1998 Apr-Jun;6(2):65-74.
- (90) Moolenaar F, Yska JP, Visser J, Meijer DK. Drastic improvement in the rectal absorption profile of morphine in man. *Eur J Clin Pharmacol* 1985;29(1):119-121.

- (91) Cole L, Hanning CD. Review of the rectal use of opioids. *J Pain Symptom Manage* 1990 Apr;5(2):118-126.
- (92) de Boer AG, Breimer DD. Hepatic first-pass effect and controlled drug delivery following rectal administration. *Adv Drug Deliv Rev* 1997 11/10;28(2):229-237.
- (93) van Hoogdalem E, de Boer AG, Breimer DD. Pharmacokinetics of rectal drug administration, Part I. General considerations and clinical applications of centrally acting drugs. *Clin Pharmacokinet* 1991 Jul;21(1):11-26.
- (94) Minto C, Schnider T. Expanding clinical applications of population pharmacodynamic modelling. *Br J Clin Pharmacol* 1998 Oct;46(4):321-333.
- (95) Perez-Urizar J, Granados-Soto V, Flores-Murrieta FJ, Castaneda-Hernandez G. Pharmacokinetic-pharmacodynamic modeling: why? *Arch Med Res* 2000 Nov-Dec;31(6):539-545.
- (96) Bijur PE, Silver W, Gallagher EJ. Reliability of the visual analog scale for measurement of acute pain. *Acad Emerg Med* 2001 Dec;8(12):1153-1157.
- (97) Drewes AM, Gregersen H. Multimodal pain stimulation of the gastrointestinal tract. *World J Gastroenterol* 2006 Apr 28;12(16):2477-2486.
- (98) Mense S. The pathogenesis of muscle pain. *Curr Pain Headache Rep* 2003 Dec;7(6):419-425.
- (99) Graven-Nielsen T. Fundamentals of muscle pain, referred pain, and deep tissue hyperalgesia. *Scand J Rheumatol Suppl* 2006;122:1-43.
- (100) Graven-Nielsen T, Arendt-Nielsen L. Induction and assessment of muscle pain, referred pain, and muscular hyperalgesia. *Curr Pain Headache Rep* 2003 Dec;7(6):443-451.
- (101) Polianskis R, Graven-Nielsen T, Arendt-Nielsen L. Computer-controlled pneumatic pressure algometry--a new technique for quantitative sensory testing. *Eur J Pain* 2001;5(3):267-277.
- (102) Polianskis R, Graven-Nielsen T, Arendt-Nielsen L. Spatial and temporal aspects of deep tissue pain assessed by cuff algometry. *Pain* 2002 Nov;100(1-2):19-26.
- (103) Olesen A, Brock C, Larsen I, Drewes A. Differential Sensitivity of Human Experimental Pain Tests to Morphine Induced Analgesia - A randomized, double-blind, placebo-controlled, cross-over study. *Journal of pain research* In press.
- (104) Olesen AE, Staahl C, Arendt-Nielsen L, Drewes AM. Different effects of morphine and oxycodone in experimentally evoked hyperalgesia: a human translational study. *Br J Clin Pharmacol* 2010 Aug;70(2):189-200.
- (105) Ebenezer GJ, McArthur JC, Thomas D, Murinson B, Hauer P, Polydefkis M, et al. Denervation of skin in neuropathies: the sequence of axonal and Schwann cell changes in skin biopsies. *Brain* 2007 Oct;130(Pt 10):2703-2714.
- (106) Kreilgaard M, Brokjær A, Simonsson U, Olesen A, Christup L, Dahan A, et al. Study design optimization of a morphine analgesia trial using PKPD modelling and simulation. *PAGE* 23 (2014) Abstr 3093 [[www page-meeting org/?abstract=3093](http://www.page-meeting.org/?abstract=3093)] .
- (107) Ness TJ, Gebhart GF. Visceral pain: a review of experimental studies. *Pain* 1990 May;41(2):167-234.
- (108) Knowles CH, Aziz Q. Basic and clinical aspects of gastrointestinal pain. *Pain* 2009 Feb;141(3):191-209.

- (109) Sengupta J, Gebhart G. Gastrointestinal afferent fibers and sensation. In: Johnson L, editor. *Physiology of the Gastrointestinal Tract* New York: Raven Press; 1994. p. 483-519.
- (110) Olesen SS, Brock C, Krarup AL, Funch-Jensen P, Arendt-Nielsen L, Wilder-Smith OH, et al. Descending inhibitory pain modulation is impaired in patients with chronic pancreatitis. *Clin Gastroenterol Hepatol* 2010 Aug;8(8):724-730.
- (111) Brock C, Nissen TD, Gravesen FH, Frokjaer JB, Omar H, Gale J, et al. Multimodal sensory testing of the rectum and rectosigmoid: development and reproducibility of a new method. *Neurogastroenterol Motil* 2008 Aug;20(8):908-918.
- (112) Brock C, Arendt-Nielsen L, Wilder-Smith O, Drewes AM. Sensory testing of the human gastrointestinal tract. *World J Gastroenterol* 2009 Jan 14;15(2):151-159.
- (113) Lundeberg T, Lund I, Dahlin L, Borg E, Gustafsson C, Sandin L, et al. Reliability and responsiveness of three different pain assessments. *J Rehabil Med* 2001 Nov;33(6):279-283.
- (114) Ravn P, Secher EL, Skram U, Therkildsen T, Christrup LL, Werner MU. Morphine- and buprenorphine-induced analgesia and antihyperalgesia in a human inflammatory pain model: a double-blind, randomized, placebo-controlled, five-arm crossover study. *J Pain Res* 2013;6:23-38.
- (115) Bergh IH, Stener-Victorin E, Wallin G, Martensson L. Comparison of the PainMatcher and the Visual Analogue Scale for assessment of labour pain following administered pain relief treatment. *Midwifery* 2011 Feb;27(1):e134-9.
- (116) Lundeberg T, Lund I, Dahlin L, Borg E, Gustafsson C, Sandin L, et al. Reliability and responsiveness of three different pain assessments. *J Rehabil Med* 2001 Nov;33(6):279-283.
- (117) Henderer J, Rapuano C. Ocular Pharmacology. In: Brunton L, Chabner B, Knollman B, editors. *Goodman & Gilman's The Pharmacological Basis of Therapeutics*. 12th ed. New York: McGraw-Hill; 2011. p. 1773-1801.
- (118) Freye E. Opioid Related Side Effects. *Opioids in Medicine: A Comprehensive Review of the Mode of Action and the Use of Analgesics in Different Pain States*. 1st ed. Dordrecht: Springer; 2008. p. 132-177.
- (119) Murray RB, Adler MW, Korczyn AD. The pupillary effects of opioids. *Life Sci* 1983 Aug 8;33(6):495-509.
- (120) Sharpe LG, Pickworth WB. Opposite pupillary size effects in the cat and dog after microinjections of morphine, normorphine and clonidine in the Edinger-Westphal nucleus. *Brain Res Bull* 1985 Sep;15(3):329-333.
- (121) Lee HK, Wang SC. Mechanism of morphine-induced miosis in the dog. *J Pharmacol Exp Ther* 1975 Feb;192(2):415-431.
- (122) Larson MD. Mechanism of opioid-induced pupillary effects. *Clin Neurophysiol* 2008 Jun;119(6):1358-1364.
- (123) Weinhold LL, Bigelow GE. Opioid miosis: effects of lighting intensity and monocular and binocular exposure. *Drug Alcohol Depend* 1993 Jan;31(2):177-181.
- (124) Baririan N, Van Obbergh L, Desager JP, Verbeeck RK, Wallemacq P, Starkel P, et al. Alfentanil-induced miosis as a surrogate measure of alfentanil pharmacokinetics in patients with mild and moderate liver cirrhosis. *Clin Pharmacokinet* 2007;46(3):261-270.

- (125) Cooper ZD, Sullivan MA, Vosburg SK, Manubay JM, Haney M, Foltin RW, et al. Effects of repeated oxycodone administration on its analgesic and subjective effects in normal, healthy volunteers. *Behav Pharmacol* 2012 Jun;23(3):271-279.
- (126) Hong D, Flood P, Diaz G. The side effects of morphine and hydromorphone patient-controlled analgesia. *Anesth Analg* 2008 Oct;107(4):1384-1389.
- (127) Kharasch ED, Hoffer C, Walker A, Sheffels P. Disposition and miotic effects of oral alfentanil: a potential noninvasive probe for first-pass cytochrome P4503A activity. *Clin Pharmacol Ther* 2003 Mar;73(3):199-208.
- (128) Kharasch ED, Walker A, Hoffer C, Sheffels P. Sensitivity of intravenous and oral alfentanil and pupillary miosis as minimally invasive and noninvasive probes for hepatic and first-pass CYP3A activity. *J Clin Pharmacol* 2005 Oct;45(10):1187-1197.
- (129) Kharasch ED, Francis A, London A, Frey K, Kim T, Blood J. Sensitivity of intravenous and oral alfentanil and pupillary miosis as minimal and noninvasive probes for hepatic and first-pass CYP3A induction. *Clin Pharmacol Ther* 2011 Jul;90(1):100-108.
- (130) Matouskova O, Slanar O, Chytil L, Perlik F. Pupillometry in healthy volunteers as a biomarker of tramadol efficacy. *J Clin Pharm Ther* 2011 Aug;36(4):513-517.
- (131) Phimmasone S, Kharasch ED. A pilot evaluation of alfentanil-induced miosis as a noninvasive probe for hepatic cytochrome P450 3A4 (CYP3A4) activity in humans. *Clin Pharmacol Ther* 2001 Dec;70(6):505-517.
- (132) Slanar O, Nobilis M, Kvetina J, Idle JR, Perlik F. CYP2D6 polymorphism, tramadol pharmacokinetics and pupillary response. *Eur J Clin Pharmacol* 2006 Jan;62(1):75-6; author reply 77-8.
- (133) Stoops WW, Glaser PE, Rush CR. Miotic and subject-rated effects of therapeutic doses of tapentadol, tramadol, and hydromorphone in occasional opioid users. *Psychopharmacology (Berl)* 2013 Jul;228(2):255-262.
- (134) Zacny JP, Lichtor JL, Klawns JM, Alessi R, Apfelbaum JL. The effects of transnasal buprenorphine on mood and psychomotor functioning in healthy volunteers. *Anesth Analg* 1996 May;82(5):931-935.
- (135) Tegeder I, Meier S, Burian M, Schmidt H, Geisslinger G, Lotsch J. Peripheral opioid analgesia in experimental human pain models. *Brain* 2003 May;126(Pt 5):1092-1102.
- (136) Lotsch J, Skarke C, Schmidt H, Grosch S, Geisslinger G. The transfer half-life of morphine-6-glucuronide from plasma to effect site assessed by pupil size measurement in healthy volunteers. *Anesthesiology* 2001 Dec;95(6):1329-1338.
- (137) Schallenberg M, Bangre V, Steuhl KP, Kremmer S, Selbach JM. Comparison of the Colvard, Procyon, and Neuroptics pupillometers for measuring pupil diameter under low ambient illumination. *J Refract Surg* 2010 Feb;26(2):134-143.
- (138) Vuong C, Van Uum SH, O'Dell LE, Lutfy K, Friedman TC. The effects of opioids and opioid analogs on animal and human endocrine systems. *Endocr Rev* 2010 Feb;31(1):98-132.
- (139) Lo Dico G, Riggio V, Cossu A, Buscarinu G, Delitala G, Stoppelli I. Role of opiates in the physiological control of prolactin in man. *Acta Eur Fertil* 1983 Nov-Dec;14(6):409-414.
- (140) Chang C, Byon W, Lu Y, Jacobsen LK, Badura LL, Sawant-Basak A, et al. Quantitative PK-PD model-based translational pharmacology of a novel kappa opioid receptor antagonist between rats and humans. *AAPS J* 2011 Dec;13(4):565-575.

- (141) Devilla L, Pende A, Morgano A, Giusti M, Musso NR, Lotti G. Morphine-induced TSH release in normal and hypothyroid subjects. *Neuroendocrinology* 1985 Apr;40(4):303-308.
- (142) Hemmings R, Fox G, Tolis G. Effect of morphine on the hypothalamic-pituitary axis in postmenopausal women. *Fertil Steril* 1982 Mar;37(3):389-391.
- (143) Delitala G, Grossman A, Besser M. Differential effects of opiate peptides and alkaloids on anterior pituitary hormone secretion. *Neuroendocrinology* 1983 Oct;37(4):275-279.
- (144) Caruhel P, Mazier C, Kunde J, Morgenthaler NG, Darbouret B. Homogeneous time-resolved fluoroimmunoassay for the measurement of midregional proadrenomedullin in plasma on the fully automated system B.R.A.H.M.S KRYPTOR. *Clin Biochem* 2009 May;42(7-8):725-728.
- (145) Bazin H, Trinquet E, Mathis G. Time resolved amplification of cryptate emission: a versatile technology to trace biomolecular interactions. *J Biotechnol* 2002 Jan;82(3):233-250.
- (146) Knott VJ. Quantitative EEG methods and measures in human psychopharmacological research. *Hum Psychopharmacol* 2000 Oct;15(7):479-498.
- (147) Constant I, Sabourdin N. The EEG signal: a window on the cortical brain activity. *Paediatr Anaesth* 2012 Jun;22(6):539-552.
- (148) Nunez PL, Srinivasan R. Electric fields of the brain: the neurophysics of EEG. 2. ed. ed. New York: Oxford University Press; 2006.
- (149) Tonner PH, Bein B. Classic electroencephalographic parameters: median frequency, spectral edge frequency etc. *Best Pract Res Clin Anaesthesiol* 2006 Mar;20(1):147-159.
- (150) Malver LP, Brokjaer A, Staahl C, Graversen C, Andresen T, Drewes AM. Electroencephalography and analgesics. *Br J Clin Pharmacol* 2014 Jan;77(1):72-95.
- (151) Dumermuth G, Ferber G, Herrmann WM, Hinrichs H, Kunkel H. International pharmaco-EEG group (IPEG). Committee on standardization of data acquisition and analysis in pharmaco-EEG investigations. *Neuropsychobiology* 1987;17(4):213-218.
- (152) Jobert M, Wilson FJ, Ruigt GS, Brunovsky M, Prichep LS, Drinkenburg WH, et al. Guidelines for the recording and evaluation of pharmaco-EEG data in man: the International Pharmaco-EEG Society (IPEG). *Neuropsychobiology* 2012;66(4):201-220.
- (153) Egan TD, Minto CF, Hermann DJ, Barr J, Muir KT, Shafer SL. Remifentanyl versus alfentanil: comparative pharmacokinetics and pharmacodynamics in healthy adult male volunteers. *Anesthesiology* 1996 Apr;84(4):821-833.
- (154) Greenwald MK, Roehrs TA. Mu-opioid self-administration vs passive administration in heroin abusers produces differential EEG activation. *Neuropsychopharmacology* 2005 Jan;30(1):212-221.
- (155) Stoermer R, Drewe J, Dursteler-Mac Farland KM, Hock C, Mueller-Spahn F, Ladewig D, et al. Safety of injectable opioid maintenance treatment for heroin dependence. *Biol Psychiatry* 2003 Oct 15;54(8):854-861.
- (156) Noh GJ, Kim KM, Jeong YB, Jeong SW, Yoon HS, Jeong SM, et al. Electroencephalographic approximate entropy changes in healthy volunteers during remifentanyl infusion. *Anesthesiology* 2006 May;104(5):921-932.
- (157) Lotsch J, Kobal G, Geisslinger G. No contribution of morphine-6-glucuronide to clinical morphine effects after short-term administration. *Clin Neuropharmacol* 1998 Nov-Dec;21(6):351-354.

- (158) Staahl C, Dimcevski G, Andersen SD, Thorsgaard N, Christrup LL, Arendt-Nielsen L, et al. Differential effect of opioids in patients with chronic pancreatitis: an experimental pain study. *Scand J Gastroenterol* 2007 Mar;42(3):383-390.
- (159) Osborne R, Joel S, Trew D, Slevin M. Morphine and metabolite behavior after different routes of morphine administration: demonstration of the importance of the active metabolite morphine-6-glucuronide. *Clin Pharmacol Ther* 1990 Jan;47(1):12-19.
- (160) Sverrisdottir E, Foster DJ, Upton RN, Olesen AE, Lund TM, Gabel-Jensen C, et al. Modelling concentration-analgesia relationships for morphine to evaluate experimental pain models. *Eur J Pharm Sci* 2014 Oct 11.
- (161) Ravn P, Foster DJ, Kreilgaard M, Christrup L, Werner MU, Secher EL, et al. Pharmacokinetic-Pharmacodynamic Modelling of the Analgesic and Antihyperalgesic Effects of Morphine after Intravenous Infusion in Human Volunteers. *Basic Clin Pharmacol Toxicol* 2014 Feb 12.
- (162) Mazoit JX, Butscher K, Samii K. Morphine in postoperative patients: pharmacokinetics and pharmacodynamics of metabolites. *Anesth Analg* 2007 Jul;105(1):70-78.
- (163) Lotsch J, Skarke C, Schmidt H, Liefhold J, Geisslinger G. Pharmacokinetic modeling to predict morphine and morphine-6-glucuronide plasma concentrations in healthy young volunteers. *Clin Pharmacol Ther* 2002 Aug;72(2):151-162.
- (164) Dahan A, Romberg R, Teppema L, Sarton E, Bijl H, Olofsen E. Simultaneous measurement and integrated analysis of analgesia and respiration after an intravenous morphine infusion. *Anesthesiology* 2004 Nov;101(5):1201-1209.
- (165) Meineke I, Freudenthaler S, Hofmann U, Schaeffeler E, Mikus G, Schwab M, et al. Pharmacokinetic modelling of morphine, morphine-3-glucuronide and morphine-6-glucuronide in plasma and cerebrospinal fluid of neurosurgical patients after short-term infusion of morphine. *Br J Clin Pharmacol* 2002 Dec;54(6):592-603.
- (166) Davies B, Morris T. Physiological parameters in laboratory animals and humans. *Pharm Res* 1993 Jul;10(7):1093-1095.
- (167) Wenk HN, Brederson JD, Honda CN. Morphine directly inhibits nociceptors in inflamed skin. *J Neurophysiol* 2006 Apr;95(4):2083-2097.
- (168) Riviere PJ. Peripheral kappa-opioid agonists for visceral pain. *Br J Pharmacol* 2004 Apr;141(8):1331-1334.
- (169) Staahl C, Christrup LL, Andersen SD, Arendt-Nielsen L, Drewes AM. A comparative study of oxycodone and morphine in a multi-modal, tissue-differentiated experimental pain model. *Pain* 2006 Jul;123(1-2):28-36.
- (170) Ross FB, Smith MT. The intrinsic antinociceptive effects of oxycodone appear to be kappa-opioid receptor mediated. *Pain* 1997 Nov;73(2):151-157.
- (171) Nozaki C, Saitoh A, Tamura N, Kamei J. Antinociceptive effect of oxycodone in diabetic mice. *Eur J Pharmacol* 2005 Nov 7;524(1-3):75-79.
- (172) Nozaki C, Saitoh A, Kamei J. Characterization of the antinociceptive effects of oxycodone in diabetic mice. *Eur J Pharmacol* 2006 Mar 27;535(1-3):145-151.
- (173) Nielsen CK, Ross FB, Lotfipour S, Saini KS, Edwards SR, Smith MT. Oxycodone and morphine have distinctly different pharmacological profiles: radioligand binding and behavioural studies in two rat models of neuropathic pain. *Pain* 2007 Dec 5;132(3):289-300.

- (174) Holzer P. Opioids and opioid receptors in the enteric nervous system: from a problem in opioid analgesia to a possible new prokinetic therapy in humans. *Neurosci Lett* 2004 May 6;361(1-3):192-195.
- (175) Drewes AM, Frokjaer JB, Larsen E, Reddy H, Arendt-Nielsen L, Gregersen H. Pain and mechanical properties of the rectum in patients with active ulcerative colitis. *Inflamm Bowel Dis* 2006 Apr;12(4):294-303.
- (176) Graversen C, Malver LP, Kurita GP, Staahl C, Christrup LL, Sjogren P, et al. Altered Frequency Distribution in the Electroencephalogram is Correlated to the Analgesic Effect of Remifentanyl. *Basic Clin Pharmacol Toxicol* 2014 Sep 24.
- (177) Le Bars D, Gozariu M, Cadden SW. Animal models of nociception. *Pharmacol Rev* 2001 Dec;53(4):597-652.
- (178) Quante M, Scharein E, Zimmermann R, Langer-Brauburger B, Bromm B. Dissociation of morphine analgesia and sedation evaluated by EEG measures in healthy volunteers. *Arzneimittelforschung* 2004;54(3):143-151.

ISSN (online): 2246-1302
ISBN (online): 978-87-7112-276-3

AALBORG UNIVERSITY PRESS